Optimisation of 2,4-D treatments for the control of common scab of potato (and related studies)

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Publications

Refereed Journal Articles

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Conference Publications

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Poster Presentations

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Abstract

Common scab is an economically important disease of potato found in most growing regions of the world. There are few practical control methods and none that are both reliable and effective. Disease is minimised through planting resistant varieties, strategic use of irrigation, seed tuber treatments and late planting. Common scab is caused by pathogenic *Streptomyces* spp. that produce thaxtomins, necrosis-causing phytotoxins that are essential for pathogenicity. Previous research had found that 2,4-dichlorophenoxyacetic acid (2,4-D), a herbicide and synthetic auxin, controlled common scab symptoms when applied to the foliage of potato, but also resulted in undesirable phytotoxic effects. It has been demonstrated that when 2,4-D is translocated to potato tubers, it suppresses thaxtomin toxicity.

This study determined optimal rates and timing of 2,4-D application for control of common scab whilst minimising phytotoxic effects of the treatments. It found that treatment of potato plants as soon as 5 days after emergence provided greater protection against common scab and greater suppression of thaxtomin toxicity in harvested tubers than treatments after tuber initiation. Rates much lower than had previously been tested were found to reduce disease and induce toxin tolerance to levels similar to that obtained with treatments at near herbicidal rates, suggesting that maximum toxicity suppression occurred at very low tuber 2,4-D levels. These very low rates did not induce any noticeable phytotoxic symptoms, nor affect harvested tuber yield or quality, and resulted in 2,4-D residue levels well below maximum residue limits in tubers at harvest. Additionally, it was found that if seed tubers were treated prior to planting, daughter tubers would have some protection from disease and show tolerance to the toxin without an additional post emergence treatment.

This study also examined genetic variation in a number of somaclonal potato lines derived from Russet Burbank that showed a higher tolerance to thaxtomin than the parent line. In prior studies, enhanced tolerance to thaxtomin through reduced cellular uptake was identified in *Arabidopsis thaliana* mutants. Fine mapping showed mutations in the gene *TXR1* were responsible for the observed phenotype. In this study, *TXR1* potato homolog genes from selected thaxtomin tolerant somaclones were cloned, sequenced and analysed for variation to determine if toxin tolerance may be associated with mutations within this gene. The parent cultivar had only two

allelic forms, whilst the thaxtomin tolerant variants possessed an additional eight unique alleles. These mutant *TXR1* alleles may have contributed toward thaxtomin tolerance in these variants.

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Chapter 1: Review of literature

1.1 Introduction

Potato (*Solanaceae tuberosum*) is an economically and socially important vegetable crop. In 2008 it was the fourth largest food crop (behind wheat, rice, and maize) with over 325 million tonnes produced worldwide. Potato is the largest vegetable crop internationally, and within Australia is the largest and highest dollar value vegetable crop, with over 140 thousand tonnes produced (Food and Agriculture Organization of the United Nations 2011). In fact, 2008 was celebrated as the International Year of the Potato (Food and Agriculture Organization of the United Nations 2008).

Common scab of potato is an economically important soil borne disease, found worldwide, and characterised by scab like lesions on the tuber surface (Loria *et al.* 1997). It is caused by a number of pathogenic *Streptomyces* spp., but predominantly by *S. scabies* (Darling 1937; Lambert & Loria 1989). There are a number of possible control methods for common scab, primarily cultural controls such as irrigation (Lapwood 1971; Lapwood *et al.* 1973), planting dates (Waterer 2002a; Wilson 2004), crop rotations and cultivar selection (Wilson 2001; Wilson *et al.* 2009; 2010; Hiltunen *et al.* 2011), as well as other methods including biological control (Liu *et al.* 1995; Neeno-Eckwall & Schottel 1999; Neeno-Eckwall *et al.* 2001; Han *et al.* 2005), but more practical and effective control methods are needed. A number of chemicals have been tested for their efficacy in controlling the disease, but many have been harmful, ineffective, or caused abnormal or reduced tuber growth (McIntosh 1973;1976;1979; McIntosh & Bateman 1979; McIntosh *et al.* 1981; 1982; 1985; 1988).

Scab causing *Streptomyces* species produce phytotoxins known as thaxtomins (King *et al.* 1989), which are necessary for pathogenicity (King *et al.* 1991; Loria *et al.* 1995; Leiner *et al.* 1996; Goyer *et al.* 1998; Kers *et al.* 2005), cause necrosis on tuber tissue (Lawrence *et al.* 1990), and allow the pathogen to enter and move between cells in the tuber (Fry & Loria 2002; Tegg *et al*. 2005). There is evidence to suggest that thaxtomins may interact with auxin within plants, with an auxin sensitive mutant of *Arabidopsis* showing increased sensitivity to thaxtomin A when compared to a wild-type (Tegg *et al.* 2005). Additionally, studies have shown that auxin in the form of 2,4-dichlorophenoxyacetic acid applied to the foliage of potato plants reduces common scab symptoms (McIntosh *et al.* 1981; Tegg *et al.* 2008; 2012; Waterer

2010), however side-effects include some tuber deformity and yield reduction. With refinement this could prove a novel control method.

1.2 Common Scab of Potato

1.2.1 Significance

Common scab of potato is one of the most economically important diseases of potato internationally and was named by Northern American growers as the fourth most important potato disease in 1991 (Loria *et al.* 1997). While the impact of the disease on yield is small, losses occur due to rejected tubers and crops for both fresh market and seed tubers (Loria *et al.* 1997; Wilson *et al.* 2010). Additionally, the disease results in decreased quality and increased processing requirements. Normal steam peeling procedures may not be sufficient to remove more deeply pitted lesioned material, requiring double peeling (Wilson *et al.* 2009). In 2004 it was responsible for losses to the Tasmanian potato industry in excess of $3.66 million AUD, approximately 4% of The Tasmanian potato industry's total value (Wilson *et al.* 2009). One of the most commonly grown potato varieties in the world, Russet Burbank, is considered moderately resistant to the disease, yet is still susceptible to deep lesions and the resultant losses under high disease pressure (Wilson *et al.* 2010).

1.2.2 Streptomyces

Streptomyces are a genus of gram-positive bacteria of the actinobacteria family. They are filamentous and spore producing, have DNA with high levels of guanine and cytosine, and the majority live in the soil and are saprophytic (Loria *et al.* 2006). *Streptomyces* are well known for producing various biologically active secondary metabolites, responsible for over two thirds of currently available, naturally produced antibiotics (Loria *et al.* 2003).

A number of species of *Streptomyces* have been found to cause common scab, including *S. turgidiscabies* and *S. acidiscabies*, but is predominantly caused by

Streptomyces scabies[1] (Lambert & Loria 1989; Loria *et al.* 2006). *Streptomyces* spp. produce spores, which are hydrophobic, allowing for their dispersal in soil water and by microscopic organisms. *Streptomyces* also produce vegetative mycelium and aerial hyphae (Loria *et al.* 2006), which allow for penetration through the soil and assists in the colonisation of, and entry into potato lenticels (Loria *et al.* 1997).

1.2.3 Symptoms and Infection

Common scab of potato is characterised by round, scab like lesions on the tuber surface, which can be superficial (Loria *et al.* 2006), or more commonly in Australia, deeply pitted (Wilson *et al.* 1999). While potato is the most economically important host of scab causing *Streptomyces*, a range of root crops are also affected by common scab, including carrot, radish and beetroot (Leiner *et al.* 1996), and it is likely that all higher plants are possible hosts.

Common scab is visually similar to another potato disease, powdery scab. Powdery scab is a disease of potato caused by the plasmodiophorid pathogen *Spongopora subterranea* f. sp. *subterranea* (Harrison *et al.* 1997). Powdery scab has been reported worldwide, but is of primary importance in cool, wet potato producing regions (Falloon 2008). Symptoms of the disease include lesions on the tuber that begin as pimple like lumps before erupting into scab like lesions filled with a powdery mass of sporeballs, called cystosori (Harrison *et al.* 1997). While powdery scab and common scab proliferate under different environmental conditions, they can affect the same tubers (Qu *et al.* 2011). There is little published research into the interaction between the two diseases.

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[1] *Streptomyces scabies* was officially renamed *Streptomyces scabiei* in 1997 to correct the grammatical error in the original name, but both *S. scabiei* and *S. scabies* are in common usage today (Lambert *et al.* 2007). To prevent confusion, *S. scabies* will be used in this thesis.

It has been demonstrated that pathogenic *Streptomyces* spp. are capable of entry through the cell wall (Clark & Matthews 1987; Joshi *et al.* 2007b). Another proposed entry point for *Streptomyces* spp. into the potato tuber are lenticels, which are sites of gas exchange through the periderm that begin to develop from stomata around the third internode (Adams & Lapwood 1978). Lenticels are more proliferated on tubers in wet soil, where the development of stomata to lenticels is favoured, whereas in dry soil deposits of suberin form (Adams 1975). Adams (1975) determined that tubers were only susceptible to infection by pathogenic *S. scabies* for a period that coincided with tuber elongation, when lenticels had yet to suberise. Lapwood & Adams (1973) determined that this susceptible period occurs when lenticels are in the 3rd and 4th internode from the apical bud, which occurs approximately four to six weeks after tuber initiation (Lapwood & Hering 1970).

Internodes are susceptible for approximately 10 days, about 1-2½ weeks after they have formed (Adams & Lapwood 1978), and individual lenticels are susceptible for approximately one week (Adams 1975). Khatri *et al*. (2011) determined that earlier formed internodes were more susceptible to infection than those formed later.

The effect of lenticel size and density on the ability of *Streptomyces* spp. to infect tubers has been debated. Darling (1937) found smaller lenticels with smaller cells in resistant cultivars. Adams (1975) disputed this, suggesting that as tubers and lenticels have differing susceptibility, and that not all lenticels of a tuber are susceptible to infection, only tubers that are susceptible should be taken into consideration. Adams (1975) found that, when selecting only susceptible tubers, no difference in lenticel size was found between resistant and non-resistant cultivars.

1.3 Thaxtomins

Scab causing *Streptomyces* spp. produce a number of phytotoxins called thaxtomins, which have been characterised by King & Lawrence (1989) as nitroindol-3-yl-containing 2,5-dioxopiperazines. There have been eleven thaxtomins characterised from pathogenic *Streptomyces* spp. (King & Calhoun 2009), and of these, *S. scabies* produces thaxtomin A in the highest quantities.

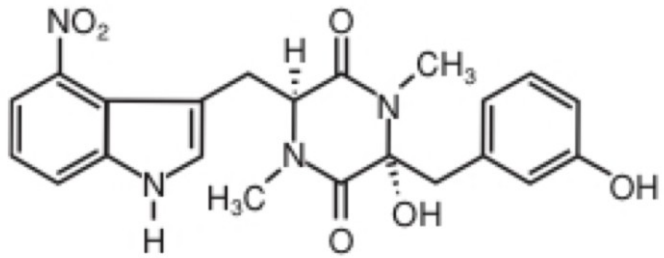

Figure 1.1 Thaxtomin A, reproduced from Loria *et al*. (2006).

1.3.1 Production

Thaxtomins were originally isolated from aseptically prepared potato tubers inoculated with *S. scabies* by King *et al*. (1989). Thaxtomins have since been produced *in vitro* in various plant extracts, demonstrating that the pathogen does not need to grow on potato tubers to produce thaxtomin. Mediums shown to facilitate thaxtomin production include oatmeal broth (Babcock *et al.* 1993), potato peel broth, fresh potato broth (Loria *et al.* 1995), and oat bran broth (Goyer *et al.* 1998).

Thaxtomin A is produced in greater levels in potato peel broth and oat bran broth, which contains the outer layers of the oat grain, than in potato flesh broth and oatmeal broth, which is primarily the endosperm (Beauséjour *et al.* 1999). This suggests that compounds found in the external parts of both potato tubers and oat grains may therefore be used by *S. scabies* in the production of thaxtomin A. Beauséjour *et al*. (1999) found that suberin enables the production of thaxtomin A when added to a minimal starch medium that, without suberin, does not facilitate thaxtomin production. Additionally, suberin has been shown to induce the production of secondary metabolites such as thaxtomin A in *Streptomyces* spp. (Lerat *et al.*

2012). Suberin is deposited in the lenticels of potato tubers in dry soil (Adams 1975), and dry soil has been shown to be more conducive to disease (Adams & Lapwood 1978). However, it has not been determined if the increase in suberin content in the lenticels of potato tubers is responsible for increased disease in dry soil.

1.3.2 Role in Pathogenicity

S. scabies has been shown to be pathogenic to a variety of monocot and dicot seedlings, causing symptoms such as reduction in shoot height, root necrosis, and root and shoot thickening. These symptoms were reproducible by directly applying thaxtomin, suggesting thaxtomins are involved in the pathogenicity of *S. scabies* (Leiner *et al.* 1996). Thaxtomin A is an important factor in the pathogenicity of *S. scabies* and the ability of a strain to produce thaxtomin A correlates with the pathogenicity of that strain (King *et al.* 2001).

Non-pathogenic strains of *S. scabies* have been found to not produce thaxtomin A *in vitro* (Loria *et al.* 1995). Goyer *et al*. (1998) found that mutant strains created with reduced levels of thaxtomin A production had reduced levels of pathogenicity, with one strain becoming non-pathogenic when its thaxtomin level was reduced by approximately 300 times. Kinkel *et al*. (1998) found a positive correlation between the amount of thaxtomin A produced in culture by a strain and the percentage of tuber surface covered by lesions, but not with the number of lesions, when inoculated with a spore suspension.

1.3.3 Biosynthesis and the pathogenicity island

Thaxtomin production by pathogenic *Streptomyces* spp. is dependent on the conserved genes *nos*, *txtR*, *txtA*, and *txtB*. *nos* encodes a nitric oxide synthase involved in the nitration of the toxin (Kers *et al.* 2004) and *txrAB* encodes for a peptide synthetase (Healy *et al.* 2000). *txtR* is involved in the regulation of thaxtomin production, encoding for a binder of cellobiose, which induces thaxtomin biosynthesis (Joshi *et al.* 2007a). While not conserved in all pathogenic *Streptomyces* spp., and not required for thaxtomin production, *nec1* encodes a protein involved in

the virulence of the pathogen. It may suppress plant defence mechanisms (Bukhalid *et al.* 1998), and is found to be secreted from the pathogen early in the infection process after pathogen entry, possibly through lenticels or directly through the cell wall (Joshi *et al.* 2007b).

Kers *et al*. discovered a pathogenicity island in the genome of several pathogenic *Streptomyces* spp. that contained both *nec1* and the thaxtomin biosynthesis pathway. This pathogenicity island can be transferred into the genome of other species, and in doing so confers pathogenicity to non-pathogenic species (Kers *et al.* 2005). This may explain the increasing range of pathogenic *Streptomyces* species (Loria *et al.* 2008).

1.3.4 Mechanism

1.3.4.1 Programmed cell death

Programmed cell death (PCD) is the hypersensitive immune response of a plant to a pathogen, where cells are killed through mechanisms built into the plant cell in order to prevent the pathogen from spreading between cells. It may occur in response to the plant sensing a pathogen-associated molecular pattern (PAMP) molecule, which are components of pathogens not present in the plant cell, typically conserved and essential to the pathogen. These PAMP molecules are recognised by pattern recognition receptor (PPR) proteins in the plasma membrane (PM). While the link is still unclear, an increase in cytosolic Ca^{2+} may occur in response to PAMP molecules being sensed by PM PPR proteins. Ca^{2+} signalling is involved in plant responses to both abiotic and biotic stresses. An increase in cytosolic Ca^{2+} occurs early in the signalling cascade involved in a cell perceiving invasion by a pathogen, and activating immune responses. Typical features of programmed cell death include the production of reactive oxygen species (ROS) such as H_2O_2 and NO, the activation of the jasmonate/salicylic acid pathways and protein kinases (Ma & Berkowitz 2011).

Ca^{2+} dependent protein kinases (CDPKs) function as Ca^{2+} sensors, and act downstream in response to increases in cytosolic Ca^{2+}. Kobayashi *et al*. (2007) found that in potato, these CDPKs could regulate the generation of reactive oxygen species

(ROS) through modifying NADPH oxidase, in response to Ca^{2+}. Increased cytosolic Ca^{2+} can initiate plant defence systems against pathogens through other signalling pathways. CDPKs have been shown to regulate gene expression in response to pathogen induced cytosolic Ca^{2+} increases. (Ma & Berkowitz 2011)

To ensure plant cells maintain a negative membrane potential, and a pH gradient across the plasma membrane (with higher acidity outside the cell), H^{+}-ATPases use energy from the hydrolysis of ATP to pump protons into the extracellular space from the cytosol. Pathogens have evolved mechanisms to target these pumps for their own means, for example to cause loosening of the cell wall through acidification to allow for penetration through them. For necrotrophic pathogens (pathogens that feed on dead tissue), stimulation of H^{+}-ATPases by the pathogen can lead to cell death (Elmore & Coaker 2011).

1.3.4.2 Thaxtomin induced cell death

Burrell (1984) found that aseptically cut potato tuber discs inoculated with *S. scabies* showed browning. Lawrence *et al*. (1990) were able to reproduce the symptoms of common scab disease by inoculating potato minitubers with cell free cultures of extractions made from common scab lesions. They determined that two of the active compounds in the extract were thaxtomin A and thaxtomin B.

In mature potato tuber tissue, thaxtomin A causes the detachment, but not the rupturing, of the plasmalemma of parenchyma cells from the cell wall (Goyer *et al.* 2000). Thaxtomin A, when applied to onion and radish seedlings caused an increase in cell volume, again suggesting a target for thaxtomin within the cell wall. When applied to suspensions of tobacco cells, the volumes of cells increased after 13.5 hours. The authors suggested this delayed reaction was due to either the thaxtomin target only being available at certain periods of the cell cycle, or that cells are only significantly affected if they are expanding at the time of exposure. Additionally, thaxtomin A inhibited cell elongation. It was concluded that thaxtomin A possibly inhibits the biosynthesis or deposition of cellulose within the cell wall (Fry & Loria 2002).

Thaxtomin A appears to trigger a biochemical cascade within the plant cell, ending in programmed cell death (PCD). The details of this cascade have not yet been fully

determined, but it has been demonstrated that a number of typical defence features of PCD, such as the production of ROS, and the activation of the ethylene/jasmonate and salicylic acid pathways (Duval *et al.* 2005), are not involved, and that PCD must occur through other mechanisms. One of the key mechanisms was demonstrated by Tegg *et al*. (2005), who showed that thaxtomin A produces an influx of Ca^{2+}, and a shift towards a net efflux of H^{+}, across the plasma membrane (PM) of root and pollen tube tissue in both *Arabidopsis* and tomato.

Errakhi *et al*. (2008) found that when this thaxtomin A induced influx of Ca^{2+} was reduced with PM calcium channel inhibitors such as La^{3+}, cell death decreased, which suggests that the Ca^{2+} influx is required to achieve thaxtomin induced cell death. Additionally, they found the when the Ca^{2+} influx was inhibited, there was a decrease in mRNA levels, and therefore the expression of the defence gene PAL1, which codes for a phenyl ammonia-lyase, a key enzyme in the phylpropanoid biosynthesis pathway, and is involved in the modulation of the production of cell wall components. The thaxtomin induced increase in PAL1 expression had previously been demonstrated by Duval *et al*. (2005).

The thaxtomin induced net efflux of H^{+} ions across the PM found by Tegg *et al*. (2005) was also found to occur by Errakhi *et al*. (2008). However, neither Fry & Loria (2002), nor Duval *et al*. (2005) observed this H^{+} efflux. Tegg *et al*. (2005) suggested that this difference was due to the 'Donnan effect' in non-buffered solutions, where the acidification of the extracellular space by H^{+} ions is equalised by a release of Ca^{2+}, masking the efflux of H^{+} ions across the PM.

Tegg *et al*. (2005) postulated that the efflux of H^{+} ions across the PM may lead to cell acidification and the displacement of Ca^{2+} ions for H^{+} ions within the cell wall, resulting in it weakening. However, Errakhi (2008) found that the efflux of H^{+} ions across the PM, and the resulting acidification of the external medium, was transient, and followed by a greater alkalization of the external medium, which involved the thaxtomin induced Ca^{2+} influx. Both Duval (2005), who found no change in cell pH, and Errakhi (2008), who observed the alkalization of the external medium, determined that there was no production of H_2O_2, a possible cause of the alkalization. As studies have observed thaxtomin to cause no change in pH (Fry & Loria 2002; Duval *et al.* 2005), external medium acidification (Tegg *et al.* 2005), and

external medium acidification then alkalization (Errakhi *et al.* 2008), we cannot yet be sure as to the effect of thaxtomin on H^+ movement across the PM in *Arabidopsis*.

Thaxtomin appears to induce an increase in the current of anions across the PM, which is consistent with other plant pathogen interactions, and in some systems requires a Ca^{2+} influx, and in others is involved in cell death. However, in *Arabidopsis* the thaxtomin induced anion current increase was transient and, when thaxtomin was combined with anion current inhibitors, cell death still occurred, suggesting that while the thaxtomin induced Ca^{2+} influx may increase the current of anions, it is not involved in the cell death (Errakhi *et al.* 2008).

A microassay of genes expression in *Arabidopsis* after treatment with thaxtomin A found that more than half the genes upregulated were also upregulated by treatment with ozone, wounding, bacterial elicitors, chitin, and H_2O_2, suggesting that these genes are part of common stress and defence signalling pathways. Thaxtomin may therefore induce cell wall damage that is sensed by the cell, which in turn causes the expression of genes related to stress and defence, resulting in PCD. The same study also observed DNA laddering, a typical feature of PCD (Duval & Beaudoin 2009).

1.3.5 txr1

A thaxtomin resistant mutant of *Arabidopsis* was identified and designated *txr1*[2] by Scheible *et al*. (2003). This mutant was found to have a decreased rate of thaxtomin uptake compared with the *Arabidopsis* wild type, which has resulted in the strain's enhanced resistance to thaxtomin. *TXR1*[3], a sequence encoding for a 116 amino acid long protein, has homologs in a number of plants, including tomato, soybean, maize, rice, and wheat. When the *txr1* gene was sequenced and compared with *TXR1* it was found to have a point mutation leading to a stop codon replacing the Arginine codon at amino acid 98. The region surrounding this codon, both within its *TXR1* homologs, and homologous genes of fully sequenced eukaryotic genomes is highly conserved,

[2] *txr1* indicates the mutant strain

[3] *TXR1* indicates the wild type allele

and the stop codon in *txr1* truncates the protein 19 C-terminal encoding amino acids early. The TXR1[4] protein did not contain organelle-targeting signals or motifs, and *TXR1* was expressed evenly through all major tissues in *Arabidopsis*. Two genes were found to have greater expression in the mutant, encoding for transport related stomatin-like proteins. The authors concluded that *TRX1* was a possible regulator of a transport mechanism, which led to the thaxtomin resistant mutant's reduced thaxtomin uptake (Scheible *et al.* 2003).

A *TXR1* homolog has also been identified in potato (Tegg 2006). While reduced sensitivity to thaxtomin appears to be involved in the common scab resistance demonstrated by some potato species (Wilson *et al.* 2009; 2010; Tegg & Wilson 2010; Hiltunen *et al.* 2011), there is currently little published work to date on the involvement of *TXR1* in common scab resistance. This thesis aims to, in part, determine if there is a relationship between thaxtomin resistant potato cultivars and mutations within *TXR1* (see Chapter 5).

[4] TXR1 indicates the protein product

1.4 Control methods

1.4.1 Targeted irrigation and pH management

Studies undertaken by Lapwood *et al*. (1971; 1973) in the United Kingdom found that irrigation applied at targeted times through the period from tuber initiation to when tubers were no longer susceptible to infection (approximately four to six weeks after tuber initiation) reduced common scab symptoms. Adams & Lapwood (1978) proposed three possible reasons for this. Firstly, as lenticel proliferation is greater in wet soil, and lenticels are a probable site of infection, infected tissue may be more readily displaced by new, uninfected tissue. Secondly, irrigation may reduce soil temperature and the availability of gases such as oxygen, thereby reducing the growth of the pathogen. Thirdly, wet soil provides higher levels of microbial competition and possible antibiotic bacteria, thereby reducing the levels of the pathogen at tuber entry sites. However, irrigation has not been found to significantly reduce scab symptoms in Australia (Wilson *et al.* 2001) and increasingly scarce and expensive water resources may make this control method less viable.

The management of soil pH to levels that are not conducive to the pathogen have been demonstrated to reduce common scab disease. Lacey & Wilson (2001) found that soils in North West Tasmania with a pH below 5.0-5.2 were not conducive to scab. Waterer (2002b) found that in a disease conducive, alkaline soil, increasing the pH above 8.5 with the addition of lime significantly reduced disease. However, the author noted that this high pH may also affect yield.

1.4.2 Delayed planting

More recently planting dates have been used to control common scab. Wilson (2004) reports that, in one season in Tasmania, planting in late spring (early November) resulted in a crop loss from common scab of 67%, which was reduced to 7% by planting in early summer (late December). Waterer (2002a) also found that early planting and late harvest increased losses from scab.

1.4.3 Soil borne and seed tuber borne inoculum

Initial studies on the role of seed tuber borne inoculum on disease, such as Lapwood (1972) and Adams & Hide (1981) found that planting infected seed tubers had no significant effect on disease levels, and suggested that soil borne inoculum was a more important factor. However, contrary to these studies, Wilson *et al*. (1999) found that inoculum introduced by visibly diseased seed tubers did significantly increase the levels of disease in daughter tubers. The importance of soil and seed tuber borne inoculum may therefore vary as a result of local conditions, such as soiltype, the pathogen population, climate and agronomic practice (Wilson *et al.* 1999). Wang & Lazarovits (2005) also found a link between the level of seed tuber borne inoculum and the incidence of scab on daughter tubers, and reported a perfect (r=1.00) correlation between the root zone population of pathogenic *Streptomyces* and disease incidence.

1.4.4 Breeding

Somatic cell selection in potato using thaxtomin A as a positive selection agent has been used to create thaxtomin A and common scab resistant varieties (Wilson *et al.* 2010; Hiltunen *et al.* 2011). Brochu *et al*. (Brochu *et al.* 2010) found that habituation of poplar cells cultures to thaxtomin A resulted in cells resistant to thaxtomin A and other inhibitors of cellulose biosynthesis, with cells having reduced levels of cellulose and increased levels of pectin. However, while the production of thaxtomin A is correlated with the pathogenicity of *Streptomyces* spp. (King *et al.* 1991), the resistance of a potato cultivar to thaxtomin A does not necessarily correlate with its resistance to common scab. Hiltunen *et al*. (2006) found that scab resistant cultivars Sabina and Nicola were tolerant to thaxtomins, and the scab susceptible cultivar Matilda was less tolerant. However, Tegg & Wilson (2010) found that scab resistant cultivars Atlantic and Russet Burbank were tolerant and sensitive to thaxtomin A respectively, and scab susceptible cultivars Tasman and Bismark were tolerant and sensitive to thaxtomin A respectively. This suggests that there are other factors involved in the level of resistance shown by cultivars to common scab disease (Tegg & Wilson 2010). However, thaxtomin tolerance is still a useful factor when screening lines that have been derived from a single cultivar, as for example in the

previously mentioned somatic cell selection used by Wilson *et al*. (2010) and other breeding programs (Hiltunen *et al.* 2011).

1.4.5 Chemical controls

1.4.5.1 Soil amendments

An early chemical control of common scab was the fungicide pentachloronitrobenzene (PCNB), also known as quintozene, which, when used as a soil amendment, gave significant control against common scab in field trials (Wilson *et al.* 1999). However, this was found to be carcinogenic, and a number of trials were undertaken in the United Kingdom (McIntosh 1973;1976;1979; McIntosh & Bateman 1979) to find a replacement chemical control. PCNB was incorporated into the soil, and so initial trials, which focused on fungitoxic soil amendments (McIntosh 1973) found that Captafol was equally effective in reducing common scab, without the yield reduction associated with PCNB. In glasshouse trials a number of quinones were also found to reduce scab, again without reducing yield. These chemicals controlled scab through their direct toxicity to *S. scabies* within the soil (McIntosh 1976).

1.4.5.2 Seed tuber treatments

As seed tuber borne inoculum can significantly increase disease severity (Wilson *et al.* 1999; Wang & Lazarovits 2005), seed tuber treatments can be used to reduce the level of inoculum on the seed tuber. The fungicides fluazinam, fenpiclonil, and flusulfamide, as well as PCNB and mancozeb, applied as either dusts or sprays to the seed tuber surface before planting, have been shown to give significant control when used on infected seed tubers. However, they did not reduce disease incidence in clean seed tubers, and so appear to only significantly affect the pathogen on the seed tuber itself. Mancozeb and PCNB both pose health risks to users, while fluazinam, fenpiclonl and flusulfamide are considered to be safer. Cement is occasionally used as a seed tuber dust treatment in Australia, but gives relatively poor control against scab (Wilson *et al.* 1999).

1.4.5.3 Foliar Sprays

In glasshouse and field trials both foliar sprays and soil amendments of daminozide, a growth regulator only weakly toxic to *S. scabies*, reduced scab incidence by half when compared to control treatments, without yield reduction. The plant hormone, gibberellic acid also reduced scab incidence but distorted tubers. Daminozide was proposed to alter the physiology of the plant in a way that decreased scab incidence, rather than interacting with the pathogen. This study demonstrated that foliar sprays, as well as soil amendments, could be used to control common scab. Additionally, it demonstrated that chemical controls could control scab without direct interaction with the pathogen, by changing the physiology of the plant itself (McIntosh 1979; McIntosh & Bateman 1979).

Ethionine, an amino acid, was also found to decrease the incidence of common scab by at least a third in glasshouse trials when applied to potato foliage. It was shown by McIntosh & Burrell (1980) to be rapidly taken into the foliage in the form of alkyl esters and distributed through all parts of the plant, with concentrations in the tuber being toxic to *S. scabies*. Burrell (1981) determined that, at the applied rates, the translocated ethionine had no effect on the metabolism of the tubers, and the higher amino acid absorption rate of *S. scabies* (up to four times greater than that of potato tuber cells), would lead to pathogen death without affecting the tuber.

A number of substituted phenoxyacetic (discussed in section 1.5.3), benzoic and picolinic acids have also been shown to control common scab to varying degrees when applied as foliar sprays (McIntosh *et al.* 1981; 1982; 1985; 1988). Both McIntosh *et al*. (1988) and Tegg *et al*. (2012) demonstrated that 2,5-dibromobenzoic acid was effective in suppressing common scab, but while McIntosh *et al.* (1988) observed both 5-chloro-2-nitrobenzoic acid and 3,6-dichloropicolinic acid to be effective as foliar sprays in controlling scab, Tegg *et al*. (2012) could not repeat these results.

1.4.6 Biological controls

Strains of *Streptomyces* that are inhibitory to *S. scabies* have been discovered and studied as possible biological controls for common scab. Two strains, isolated from the lenticels of symptom free potato tubers grown in scab suppressive soil, *S. scabies* PonR and *S. diastatochromogenes* PonSSII, have been shown to be antagonistic towards pathogenic *S. scabies* when inoculated into common scab infested soil. Both strains produce antibiotics lethal to pathogenic *S. scabies* (Liu *et al.* 1995). However, whilst strains have been shown to produce antibiotics *in vitro,* it is not known if antibiotics are produced once in the soil rhizosphere, and therefore whether antibiotic production is involved in the biological control of common scab, or whether competition against pathogenic *S. scabies* for nutrients and sites of infection on lenticels plays a greater role (Ryan & Kinkel 1997). Research focusing on microbial communities in soils has demonstrated that common scab suppressive soils have a higher level of antagonistic strains than in soils conducive to the disease (Meng *et al.* 2012; Rosenzweig *et al.* 2012).

Spontaneous mutants of inhibitory *Streptomyces* strains, which are less inhibitory to *S. scabies in vitro* than their parent strains, were isolated by Schottel *et al*. (2001) and used to determine the role that antibiotic production plays in biological control. It was found that these strains, with reduced inhibitory ability *in vitro*, still gave high levels of disease suppression in soil. This suggests that there may not be a strong correlation between *in vitro* inhibition and disease suppression, and that factors other than antibiotic production may be important in biological control of *S. scabies* (Schottel *et al.* 2001). These factors may include competition for, and exclusion from infection sites on tuber lenticels, changes in the populations of, and dynamics between the pathogen and antagonistic soil microorganisms (Neeno-Eckwall *et al.* 2001).

Multiple mutants of pathogenic *S. scabies* resistant to the antibiotics produced by the two strains studied by Lui *et al*. (1995) have since been discovered. The antibiotic resistant mutants are less pathogenic and caused less disease than their parent strains, and two of the mutants identified had lost the ability to produce thaxtomin and therefore to cause disease. The relationship between spontaneous mutations leading to antibiotic resistance, and reduction in thaxtomin production and pathogenicity is

not known. The authors, however, suggested that these mutations might have the effect of reducing the pathogenicity of populations of *S. scabies* in the presence of antibiotic producing inhibitory strains (Neeno-Eckwall & Schottel 1999).

Some bacteria, including those of the genus *Streptomyces*, have been shown to utilise thaxtomin A as a source of carbon and nitrogen. Two non-pathogenic strains of *Streptomyces* spp., EF-50 and EF-73, phylogenetically related to *S. mirabilis*, and also sharing physiological traits with pathogenic *S. scabies*, have been shown to possess this ability to utilize thaxtomin A. These two strains were shown to be not inhibitory to the growth of *S. scabies in vitro*, yet when inoculated onto tubers, reduced common scab levels in daughter tubers to 40%, from 70% in their absence (Doumbou *et al.* 1998).

1.5 Auxin Induced Resistance

1.5.1 Chemical induced resistance

A resistance to pathogens can be induced in plants where, through the production of compounds or changes in physiology, the plant becomes resistant either to a specific pathogen or to an attack in general. This resistance can be induced in a number of ways, including coming into contact with the pathogen itself, through wounding by herbivores, or through contact with exogenous chemical compounds. Resistance can either occur in the directly affected cells, or be systemic (Heil & Bostock 2002).

The plant responses involved in plant cell death (discussed in section 1.3.4), such as the jasmonic acid and salicylic acid pathways, ROS, and other phytohormones such as abscisic acid, are activated by the aforementioned inducers of resistance, and are involved in the signalling of defence mechanisms such as the production of metabolites within the plant (Halim *et al.* 2006; Ballaré 2011). This response has been demonstrated in potatoes. Andreu *et al*. (2006) found that applications of DL-3-aminobutyric acid (BABA) and fosetyl-aluminium to potatoes prior to inoculation with *P. infestans*, the causal agent of late blight, increased the defence mechanisms of the plant in response to the pathogen, and decreased disease symptoms.

1.5.2 Herbicide and indole induced resistance

Herbicides have previously been shown to induce resistance in plants against various plant pathogens. Resistance to vascular wilt diseases caused by *Fusarium* and *Verticillium* species has been achieved in tomato and eggplant by pre-treating seedlings with dinitroaniline herbicides, with the herbicides causing an accumulation of fungitoxic chemicals within the pre-treated plants, but only after inoculation with the pathogens. This suggests that the dinitroaniline herbicides induced a resistance in the plant to the pathogens, rather than directly causing the production of the fungitoxins (Grinstein *et al.* 1984).

Cohen *et al*. (1996) demonstrated resistance to Fusarium wilt in melons induced by the herbicide chloroacetamide Acetochlor. While both treated and untreated plants had similar colonisation by the pathogen, those that had been treated with Acetochlor had reduced disease, suggesting the disease reduction was not due to a fungicidal effect. A possible mechanism by which resistance to Fusarium Wilt is induced by the dinitroaniline herbicides and Acetochlor is the increased levels of amino acids found in melon seedlings treated with these herbicides (Starratt & Lazarovits 1999). Indole-3-acetic acid (IAA), a plant hormone, and tryptamine and tryptophan, indole-related chemicals, have been shown to induce resistance in barley against Rice Blast Fungus, *Magnaporthe grisea*. The suggested mechanism by which resistance is induced is the increased activity of enzymes related to fungal resistance in plants, such as phenylalanine ammonia-lyase, peroxidase, and chitinase, and increased production of H_2O_2, in response to the IAA, tryptamine, and tryptophan (Ueno *et al.* 2004).

1.5.3 Auxin induced resistance

Herbicides and related compounds have also been tested for their ability to control common scab of potato. Foliar applications of phenoxyacetic acid based sprays, including 3,5-dichlorophenoxyacetic acid (3,5-D), and the herbicide 2,4-dichlophenoxyacetic acid (2,4-D) have been shown to reduce the development of common scab symptoms (McIntosh *et al.* 1981; 1982). McIntosh *et al*. (1981) found that 2,4-D and 3,5-D reduced common scab by 45% and 87% respectively compared to untreated controls. While 3,5-D showed greater disease control, plants sprayed

with 3,5-D had decreased yield, higher numbers of tubers per plant (thereby decreasing individual tuber weight) and showed a large increase in the number of deformed tubers. The synthetic auxin 2,4-D was also shown to reduce yield to a greater extent than 3,5-D, yet it did not cause an increase in tuber number or deformity. Earlier sprays of 3,5-D were found to be more effective in reducing scab than later sprays but also increased the percentage of deformed tubers, with sprays at tuber initiation giving the greatest control. However, field trials (McIntosh *et al.* 1982) of 3,5-D resulted in a dramatically lower disease reduction of 30%, compared to glasshouse trials where 90% reduction was achieved. More recently Tegg *et al*. (2008; 2012) and Waterer (2010) have repeatedly demonstrated that 2,4-D applied to the foliage of potato plants at concentrations similar to and above those used by McIntosh *et al*. (1981) suppresses common scab.

1.5.4 Mechanisms

The levels of 2,4-D found within the potato tuber as a result of this treatment do not affect either the growth of *S. scabies* nor its ability to produce thaxtomin A (McIntosh *et al.* 1981; Tegg *et al.* 2008). It was proposed that 3,5-D altered the metabolism of the tuber preventing scab development (McIntosh *et al.* 1981). However, Tegg *et al*. (2008) demonstrated that the applied 2,4-D did not significantly change the tuber in aspects believed to be critical for infection by *S. scabies*, such as lenticel dimensions or number, or periderm thickness and structure.

Burrell (1984) demonstrated that potato discs soaked in 3,5-D and 2,4-D showed reduced browning when compared to control discs and proposed that the reduction in common scab symptoms was due to the inhibition of phenolic biosynthesis involved in browning. Both foliar applied 3,5-D and 2,4-D are translocated to tubers, but 3,5-D is translocated more quickly and metabolised at a slower rate than 2,4-D, suggesting that the greater scab control from 3,5-D may be due to the increased amounts within the tuber (Burrell 1982).

Tegg *et al*. (2008) found treatments of 2,4-D suppressed the toxicity of thaxtomin A within the tuber tissue, with tubers from treated plants showing less necrosis in the presence of thaxtomin A, than those from control plants. Tegg *et al*. (2012)

demonstrated that there was a correlation between the level of control of common scab by of a range of chemicals, when applied to the foliage of potato plants, and the resultant thaxtomin toxicity to tubers. This suggested that the ability to suppress thaxtomin toxicity is an important factor in controlling common scab.

An auxin sensitive mutant of *Arabidopsis* (*ucu2-2*/*gi2*) has been shown to have the H^+ flux patterns of its roots affected to a greater extent than those of an *Arabidopsis* wildtype in response to applied thaxtomin A, as well as being observed to have an increased susceptibility to thaxtomin (Tegg *et al.* 2005). This again suggests that there is a possible interaction between auxin and thaxtomin A. Tegg *et al*. (2005; 2008) postulated that thaxtomin A may be acting on auxin receptors, and that increased auxin within the cells may provide competition with thaxtomin A, for these receptors, reducing the effect of thaxtomin A.

However, Duval & Beaudoin (2009) found that very few auxin responsive genes are upregulated in response to thaxtomin A in *Arabidopsis*, and as such argue that thaxtomin A does not act on auxin receptors. Additionally they observed that isoxaben, another inhibitor of cellulose synthesis, similarly enhanced root formation, despite being structurally different to thaxtomin A. The authors suggested that it may be the effect of thaxtomin on the cell wall, modifying auxin transport and increasing the sensitivity of the auxin sensitive *Arabidopsis* mutant to thaxtomin, although no effect on auxin transport from the inhibition of cellulose synthesis has been shown (Duval & Beaudoin 2009). Confirmatory evidence of this has been obtained in our laboratory, whereby thaxtomin A resistance in *Arabidopsis* has been associated with enhanced resistance to an auxin transport inhibitor, 1-NPA (R. Tegg, pers. comm.).

Errakhi (2008) found that addition of IAA, an auxin, had no effect on thaxtomin induced changes in external pH in *Arabidopsis*, and suggested that thaxtomin A and IAA do not interact at this level.

1.5.5 Commercial use of 2,4-dichlorophenoxyacetic acid

In North America 2,4-D is a registered chemical for potato (Waterer 2010) where it is used to enhance the red skin colour of fresh market potatoes. Waterer (2010) found

that applied 2,4-D did significantly change the skin colour of two red skinned cultivars, and reduced tuber size, but had no significant effect on yield or tuber shape. In addition to the changes in tuber colour and size, the 2,4-D treatments also had an effect on the incidence of common and powdery scabs, with the 2,4-D treatments showing decreased common scab symptoms but increased powdery scab symptoms.

In Australia 2,4-D is registered for use in potato crops for weed control, but is not registered for use directly onto potato plants for disease control (Nufarm Australia Limited 2011). The Australian maximum residue level (MRL) for 2,4-D in potatoes is currently 0.1g/kg (100ng/g) of tuber fresh weight (TFW) (Commonwealth of Australia 2011). Tegg *et al*. (2008) found that, at foliar spray rates suppressive to common scab, levels of 2,4-D within the tuber were between 255 and 463ng 2,4-D/g TFW two weeks after treatment, and between 193 and 304ng 2,4-D/g TFW 6 weeks after treatment. While this is above the Australian MRL, these values are taken from tubers grown in pot trials, and prior to normal harvest times. Tubers grown in field conditions and harvested after plant senescence are likely to contain lower levels of 2,4-D. Therefore, further experimental work needs to be undertaken to determine likely residue limits in commercial crops.

Thesis Aims

This thesis primarily aims to build on the work of McIntosh *et al*. (1981; 1982), Tegg *et al*. (2008; 2012) and Waterer (2010), in their research associated with the control of common scab through foliar treatments of 2,4-D.

This thesis describes studies that aimed to determine:

1) the optimal period of application of 2,4-D foliar treatments to provide the greatest common scab control, and the effect of these treatments on the levels of 2,4-D in tubers at harvest;
2) the effect of single and multiple treatments on disease control when applied at various stages of plant development;
3) the relationship between the rate of 2,4-D application and resultant disease control and the level at which 2,4-D is no longer effective at controlling disease; and
4) if 2,4-D applied to seed tubers can effectively control common scab in a similar way to foliar treatments.

Additionally, this thesis aimed to determine if mutations with the potato homolog of *TXR1*, the gene identified by Scheible *et al*. (2003) to be responsible for a thaxtomin resistant phenotype in *A. thaliana*, may be responsible for the thaxtomin resistance of potato variant lines created by somatic cell selection which used thaxtomin A as a positive selection agent (Wilson *et al.* 2010).

This thesis has been written in the form of individual manuscripts for each chapter, which is the preferred format of the School of Agricultural Science. As a result, some elements of repetition between chapters may occur.

Chapter 2: Determination of optimal timing of the foliar application of 2,4-D for common scab control in potato

2.1 Abstract

Application of 2,4-dichlorophenoxyacetic acid (2,4-D) has been shown to be an effective control method for common scab of potato, but earlier research suggested that it can be ineffective in controlling disease if applied after tubers have begun to be susceptible to the disease. Treatments had previously been targeted to the start of the susceptible period, following tuber initiation. This study examines the effect of a range of application dates on disease control, in both Russet Burbank and Desiree cultivars, and presents the results of two field trials and two pot trials. The data obtained show that applications of 2,4-D as early as 5 days after plant emergence gives greater disease control than later treatments targeted to tuber initiation. In addition, these early treatments provide sufficient material to the tuber to induce resistance that lasts throughout the period in which the tuber is susceptible and result in levels of 2,4-D at harvest both well below the Australia maximum residue level, and lower than that of treatments applied later in the growing season.

2.2 Introduction

Common scab is a globally important soil borne disease of potato characterised by scab-like lesions on the tuber surface (Loria *et al.* 2006). It is caused by a number of pathogenic *Streptomyces* spp., most notably *S. scabies*. Pathogenic *Streptomyces* spp. produce a number of phytotoxins, called thaxtomins (King *et al.* 1989) which are essential for disease development. Common scab symptoms can be reproduced through the direct application of thaxtomins (Lawrence *et al.* 1990; Leiner *et al.* 1996). The capacity of *Streptomyces* spp. to produce thaxtomin A is correlated with their pathogenicity (Loria *et al.* 1995; Goyer *et al.* 1998; King *et al.* 2001; Kers *et al.* 2005). Thaxtomin production is therefore an important pathogenicity factor, and the reduction in its phytotoxic effect may be one method of controlling common scab.

There is no single method for effectively controlling common scab. Disease control is currently attempted through a combination of strategies, including the management of planting dates (Waterer 2002a; Wilson 2004), the planting of moderately resistant cultivars (Groza *et al.* 2005; Pasco *et al.* 2005; Wilson *et al.* 2010; Hiltunen *et al.* 2011) and strategic use of irrigation during early tuber development (Lapwood *et al.* 1973), the latter used extensively in the United Kingdom. Irrigation has not been as effective in Australia for management of common scab (Wilson 2004), and increasingly scarce and expensive water resources worldwide require other management strategies to be sought. Despite various adoptions of these practices, common scab occurs frequently and results in substantial losses around the world.

Treatment of potato seed tubers with fungicides or other biocides can be useful in some circumstances (Wilson *et al.* 1999), but there are no currently available effective soil-applied chemical treatments. Pentachloronitrobenzene (PCNB) was a soil-applied fungicide that did provide reasonable disease suppression and was widely used (Davis *et al.* 1974; Hooker 1981), however, the material is a known carcinogen and its use has been withdrawn from most potato production regions around the world. After the banning of PCNB in the United Kingdom, a series of trials were run between 1973 and 1978 to test a range of alternate chemicals for their ability to control common scab of potato in order to find a PCNB substitute. The trials initially focussed on materials incorporated into the soil with a number of

chemicals were shown to be as effective as PCNB, but they displayed varying degrees of phytotoxic effects on the plant (McIntosh 1973;1976). Later trials evaluated foliar applied chemical treatments, and found 2,4-dichlorophenoxyacetic acid (2,4-D) and 3,5-dichlorophenoxyacetic acid (3,5-D) to be highly effective in controlling disease in glasshouse trials. However both materials produced significant phytotoxic effects on treated plants, including reduced yield, an increased numbers of small tubers, and tuber deformation. Further to these phytotoxic effects, control of common scab by 3,5-D dropped dramatically from glasshouse trials (90% reduction in disease severity) to field trials (30% reduction). As such, it was not considered to be a viable control method by the authors (McIntosh *et al.* 1981; 1982).

The mechanism by which 2,4-D, a synthetic auxin and potent herbicide, controls common scab was not determined by McIntosh and his colleagues. Effective levels of 2,4-D did not affect the growth of the pathogen (McIntosh *et al.* 1981; Tegg *et al.* 2008), or its ability to produce thaxtomin A (Tegg *et al.* 2008). Studies by Tegg *et al*. (2008; 2012) demonstrated a strong relationship between disease levels and the tolerance of tuber tissues to thaxtomin induced toxicity, in plants that have been treated with 2,4-D. Furthermore, Tegg *et al.* (2012) found that a range of other foliar applied chemicals which also reduce common scab similarly increase tuber thaxtomin tolerance. They showed a significant negative correlation between the extent of disease expressed and the reduction in necrosis following thaxtomin treatment in tubers harvested from treated plants. This suggested 2,4-D and these other materials may operate by reducing sensitivity of potato tubers to damage by thaxtomin, and by this reducing the invasive capacity of the pathogen.

In attempts to optimise the application of 3,5-D to obtain the greatest disease suppression, McIntosh *et al*. (1981) found that earlier applications of 3,5-D in the plants development increased the efficacy of disease control, but it also increased the material's phytotoxic effects on the potato plant, further decreasing yield and tuber quality. Through the use of strategically applied irrigation treatments, which were known to suppress common scab, Lapwood *et al*. (1973) were able to confirm that tubers were only susceptible to infection by pathogenic *Streptomyces* spp. for a defined period, coinciding with tuber elongation, and presence of immature (non-suberised) lenticels (Lapwood *et al.* 1973; Khatri *et al.* 2011; Adams 1975). This

occurs approximately four to six weeks after tuber initiation, when stomata transform into lenticels (Lapwood & Hering 1970). Initial Australian studies examining the use of 2,4-D and other chemicals for the control of common scab targeted this critical infection period with treatment applications, scheduling treatments for 2 weeks after tuber initiation (Tegg 2006; Tegg *et al.* 2008). In these studies, tuber initiation was estimated by visual assessment of plants for hook development on underground stolons, and given tuber initiation for any potato plant is asynchronous with tubers initiating periodically during plant development, accurate timing of treatments to cover this "infection window" was difficult. There was a possibility that treatments may have been applied after some (or perhaps many) developing tubers had already entered the critical infection period, and thus would have failed to protect these tubers from infection. Furthermore, timing of treatment to coincide with the period of susceptibility does not allow for variation in the rate or efficiency in the translocation of the applied chemicals to the tubers. Burrell (Burrell 1982) found that after 1 day only 0.3% of 2,4-D applied to foliage had been translocated to tubers, and after 4 days it increased to only 2-3%. As these treatments have no biocidal effect against the pathogen, it is not believed that they have any effect on disease development once infection has occurred.

Previous studies (Tegg 2006; Tegg *et al.* 2008) used multiple applications staggered throughout the infection period in order to increase the duration of treatment to protect the tuber for the entire period it is susceptible to infection. Tegg *et al.* (2008) found that multiple applications provided greater control against common scab than single applications, when the initial application was targeted to the beginning of the infection window.

This chapter describes studies that aimed to determine the optimal timing for application of 2,4-D foliar treatments to provide the greatest common scab control; to determine the effect of these treatments on the levels of 2,4-D in tubers at harvest; and to further examine the effect of single and multiple treatments on disease control when applied at various stages of plant development.

2.3 Materials and Methods

2.3.1 Inoculum preparation for pot trials

Pathogenic *Streptomyces scabies* strain G#20, initially isolated from a common scab infected tuber harvested from north-west Tasmania in 1990, was used in these experiments. The isolate was grown on 10mL ISP2 (Shirling & Gottlieb 1966) agar slopes (10g/L malt extract, 4g/L yeast extract, 4g/L glucose, 12g/L agar, pH 7.3) until sporulation. Colonised agar slopes were then aseptically transferred to a sterilised mixture of 120g vermiculite and 500mL SAY solution (20g/L sucrose, 1.2g/L *L*-asparagine, 0.6g/L K_2HPO_4, 10g/L yeast extract, pH 7.2) (Labruyère 1971). The inoculum was incubated in the dark at 24ºC until profuse sporulation was observed at around 14 days.

Approximately 1L of colonised vermiculite inoculum was added to 25L of potting mix containing sand, peat and composted pine bark at a ratio of 10:10:80, at pH 6.0, premixed with Osmocote 16:3.5:10 N:P:K resin coated fertiliser (Scotts Australia Pty Ltd.) at the rate of 6kg/m^3, and thoroughly mixed using a cement mixer. The inoculum containing potting mix was then used to fill plastic planter bags of 5L capacity (200 x 200mm, Botany Horticultural, Queensland, Australia).

2.3.2 Planting material

A fortnight prior to planting, potato tubers of the varieties Russet Burbank and Desiree were removed from cold storage (4ºC), where they had been stored since harvest approximately six months before. For the pot trials, tubers were disease-free mini-tubers from glasshouse grown tissue-culture plantlets. In pot trial #1 the tubers were cut into approximately 15g pieces and left to suberise at room temperature 7 days before planting. In pot trial #2 15g tubers were used and were planted whole. For the both field trials, tubers were visibly clean, certified seed tubers.

2.3.3 Preparation of 2,4-D treatments

2.3.3.1 Pot trials

2,4-D solutions, were prepared by dissolving 200mg and 100mg of crystalline 2,4-D (Sigma Aldrich, St. Louis, USA) in approximately 10mL of 70% ethanol over heat. These solutions were then made up to 1L with warm water to produce 200mg/L and 100mg/L solutions, respectively. A control treatment of warm water only was used. Warm water was used for all treatments to assist in keeping the 2,4-D in solution. Tween-80 was added at a rate of 0.5g/L to all 2,4-D and control treatments as a wetting agent to assist foliar application.

2.3.3.2 Field trials

As the spray treatments would be applied from a backpack spraying rig in close proximity to other crops, and in accordance with licenses, the less volatile amine form of 2,4-D was used to minimise the chance of damage to nearby horticultural crops. The commercially available herbicide Amicide 625 (Nufarm Ltd., Victoria, Australia) with 625g/L 2,4-D present as dimethylamine and diethanolamine salts was used, and it was ensured that an equivalent amount of the active 2,4-D molecule was present compared with that used in the pot trials.

To prepare the spray treatments, 2.2mL or 550µL of Amicide 625 was dissolved in 1L or water, with 0.5g of Tween-80 as a wetting agent. This solution was then mixed with 9L of water within the backpack spraying rig to make either a 220µL/L solution or 55µL/L solution. This was determined to be the equivalent of a 100mg/L 2,4-D or 25mg/L 2,4-D solution respectively.

The spray rig was washed with water between the applications of different treatments. Where applicable, treatments were applied from the lowest rate to highest. The control treatment was applied with a clean handheld sprayer. The spray was applied to the foliage until run off occurred.

2.3.4 Pot trials

Two pot trials testing varied timing and frequency of 2,4-D foliar treatments were conducted.

2.3.4.1 Pot trial #1 (2009)

The first pot trial was planted on 30th January, 2009. The two potato varieties used possessed moderate resistance (Russet Burbank) and moderate susceptibility (Desiree) to common scab. Plants emerged on 8th February 2009. There were 15 different 2,4-D spray treatments, grouped in four distinct application strategies.

1. "Single" – a single treatment of 200mg/L 2,4-D applied at 10, 20, 30, 40 or 50 days after emergence (DAE).
2. "Double" – two treatments, each of 200mg/L 2,4-D, the first applied at 10, 20, 30, 40 or 50 DAE, the second applied 10 days after the first application (i.e. at 20, 30, 40, 50 and 60 DAE respectively), with a total of 400mg/L 2,4-D applied.
3. "Double (half rate)" – two treatments, each of 100mg/L 2,4-D, the first applied at 10, 20, 30, 40 or 50 DAE, the second applied 10 days after the first application (i.e. at 20, 30, 40, 50 and 60 DAE respectively), with a total of 200mg/L 2,4-D applied.

There were three control treatments, one applied as a single spray at 10 DAE, and two applied as double sprays at 10 and 20 DAE. Each treatment was applied to five pots of each variety (180 pots in total), with pots arranged in a completely randomised design. Prior to application, plants to be treated were removed to a separate area to prevent spray drift onto plants of different treatments. The sprays were applied to the foliage as a fine mist until run-off occurred. The spray was allowed to dry before the plants were returned to their position in the glasshouse.

All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease. No other pesticides were applied. Plants were grown under glasshouse

conditions, with the temperature maintained at 25-30ºC. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to each pot to prevent exposure of developing tubers to sunlight. Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 25th May, 2009. Tubers were stored at 4ºC in plastic netted bags.

Soil was brushed from tubers, which were then assessed for common scab severity using the methods described in section 2.3.6. Tuber number and mass (fresh weight) were measured. Any tuber disfigurements were noted. Sensitivity to thaxtomin A was assessed in two tubers from each pot. Tubers without obvious lesions were chosen and assayed following the method described in section 2.3.7.

2.3.4.2 Pot trial #2 (2010)

The second pot trial was planted on 19th January, 2010. Treatments and application times repeated those used in pot trial #1. Plants emerged on 1st February, 2010. Plants were maintained as per pot trial #1, but were grown outside on a concrete slab and subject to natural weather events and temperatures. Irrigation was applied as required ensuring soil dried between watering events. Tubers were harvested, following plant senescence, on 1st May, 2010. Disease, yield and toxin sensitivity assessments were as per pot trial #1.

2.3.5 Field trials

Two field trials further tested varied timing and frequency of applications of 2,4-D.

2.3.5.1 Field trial #1 (2008/09)

The site of field trial #1 was located upon the property of Mr. Stewart McGee at Bishopsbourne in North West Tasmania, and had a brown clay soil and had been sown with a processing potato crop in the previous season. The surrounding area was sown with a processing green pea crop in the trial season. The trial was planted on 21st October, 2008. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants, and plots consisting of two subplots, one of each variety. To ease planting the subplots were not randomised within each plot. There were four replicates. A visually distinct variety was planted as a buffer at the edge of the trial, between each treatment row, and between treatment plots within each row.

There were 15 2,4-D spray treatments, grouped in three distinct application strategies:

1. "Single" – a single treatment of 100mg/L 2,4-D applied at 10, 20, 30, 40, 50, or 60 DAE.
2. "Double" – two treatments, each of 100mg/L 2,4-D were applied, the first at 10, 20, 30, 40, or 50 DAE, and the second 10 days after the first treatment (i.e. at 20, 30, 40, 50, and 60 DAE respectively).
3. "Triple" – three treatments, each of 100mg/L 2,4-D were applied, the first at 10, 20, 30, or 40 DAE, the second 10 days after the first treatment (i.e. at 20, 30, 40, and 50 DAE respectively), and the third 20 days after the first treatment (i.e. at 30, 40, 50, and 60 DAE respectively).

There was one control treatment for each application strategy, applied at 10 DAE for the single treatment, at 10 and 20 DAE for the double treatment, and at 10, 20 and 30 DAE for the triple treatments.

The average emergence date was estimated to be November 30, 2008. Tubers were harvested on 2nd April, 2009. The trials were watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required. After senescence the trials were sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4ºC prior to assessment.

Before assessment the tubers were washed to remove soil and were then weighed to determine yield for each plot (the combined tubers from five plants). Each tuber was then assessed for common scab severity (DCS and LDS), and in field trial #1, powdery scab severity (DCS), using the scoring methods described in section 2.3.6. Tuber thaxtomin sensitivity was assessed using the method described in section 2.3.7 modified so that tubers were taken from replicates 1, 2 and 3 of the trial. Each replicate provided tubers for three Petri dishes (one tuber per dish), and eight filter paper discs were spread across the slices within a single Petri dish. Therefore, for each treatment, 72 filter paper discs spread over nine tubers in nine Petri dishes were used.

2.3.5.2 Field trial #2 (2009/10)

The site of field trial #2 was located upon *Forester Lodge*, the property of Mr. Wilton Geale at Waterhouse in North East Tasmania and had been sown with a processing potato crop in the previous season. The soil was predominately sandy. The surrounding area was sown with an oil seed crop in the trial season. The trial was planted on 17th November, 2009. The potato varieties, arrangement and replication repeated that used in field trial #1.

There were 22 2,4-D spray treatments, grouped in two distinct application strategies:

1. "Single" – a single treatment of either 25mg/L or 100mg/L 2,4-D, each applied at 5, 10, 20, 30, 40, or 50 DAE
2. "Double" – two treatments of either 25mg/L or 100mg/L 2,4-D, each applied at 5, 10, 20, 30, or 40 DAE, with a second application at 10, 20, 30, 40, and 50 DAE respectively.

There were two control treatments for each application strategy; two applied at 10 DAE for the single treatment, and two applied at 10 and 20 DAE for the double treatment. The average emergence date was estimated to be 10th December, 2009. Tubers were harvested on 8th April, 2010. Trial maintenance and harvest were as per field trial #1. Disease, yield and toxin sensitivity assessments were as per field trial #1. The levels of 2,4-D within selected tubers at harvest were quantified using the method described in section 2.3.8.

2.3.6 Tuber disease assessment

Each tuber was scored for presence of any disease lesions (disease incidence) and for two measures of disease severity: mean tuber surface coverage with lesions and depth of the deepest lesion. The following methods were used.

Using the chart prepared by Richardson & Heeg (1954), tubers were assessed visually, and the percentage surface area covered by disease was converted using the following scale and recorded as a disease cover score (DCS):

DCS	Percentage
0	no disease
0.5	0-1%
1	1-5%
2	5-10%
3	10-30%
4	30-50%
5	50-70%
6	70-100%

Disease severity was also determined as the depth of the deepest lesion on each tuber, using the following scale modified from Bjor & Roer (1980) and recorded as a lesion depth score (LDS):

LDS	Lesion Depth
0	no disease
1	superficial/slightly raised
2	1-2mm deep
3	2-3mm deep
4	>3mm deep

2.3.7 Tuber thaxtomin sensitivity

Tuber thaxtomin sensitivity was measured using the following method of Tegg *et al*. (2008). Using a hole-punch, disks of 6mm diameter were cut from filter paper (Whatman No. 1) and then autoclaved for 20 minutes. The disks were soaked in a solution of purified thaxtomin A (Wilson *et al.* 2009) (14µM for Desiree, 7µM for Russet Burbank) for 1 hour and then air dried in a laminar flow cabinet. Selected tubers harvested from the pot trials and stored for approximately 2 weeks at 4ºC were surface sterilised in 0.5% sodium hypochlorite for 10 minutes and then, under aseptic conditions, cut into 0.5cm slices, with the slices from each end of the tuber being discarded. The tuber slices were then placed into 90mm Petri dishes, lined with filter paper (Whatman No. 1) moistened with 2mL sterile, distilled water. Depending on tuber size, 2-4 filter paper disks were placed onto the cut surface of each tuber slice, with a total of 10 disks per Petri dish. The discs were moistened with 10µL sterile distilled water to ensure their attachment to the surface of the tuber slice. The tuber slices were then incubated in the dark at 24ºC for 7 days.

After 7 days the filter paper discs were removed from the cut surface of the tuber and visible necrosis underneath was scored using the following scale (Figure 2.1):

Score	**Criteria**
0	no necrosis
0.5	very sparse flecks
1	few light brown flecks
1.5	few dark brown flecks
2	light brown flecks in a circle
2.5	dark brown flecks in a circle
3	light brown necrosis
3.5	dark brown necrosis
4	black necrosis

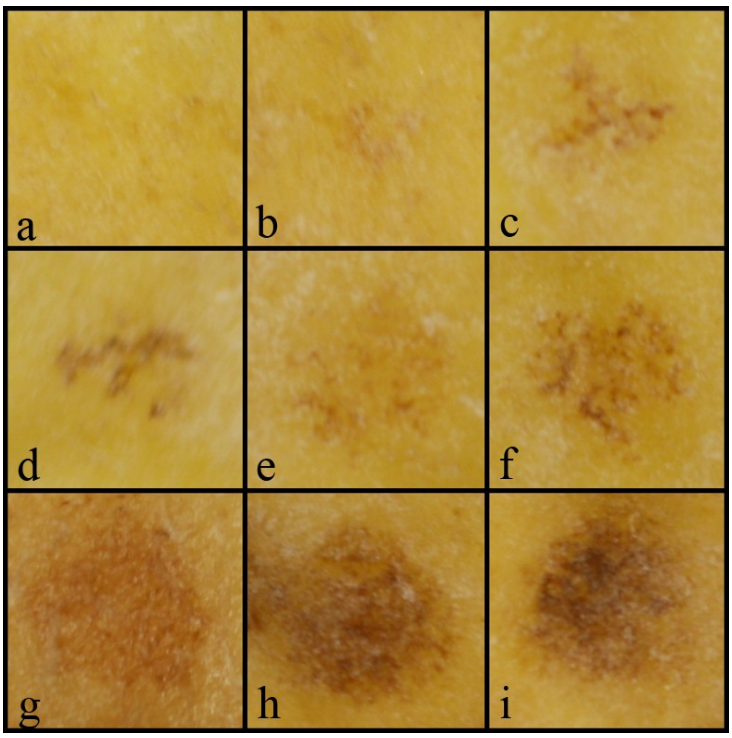

Figure 2.1 Examples of necrosis scored using the scale:
a. 0 = no necrosis **b.** 0.5 = very sparse flecks
c. 1 = few light brown specks
d. 1.5 = few dark brown specks
e. 2 = light brown flecks in a circle
f. 2.5 = dark brown flecks in a circle
g. 3 = light brown necrosis
h. 3.5 = dark brown necrosis
i. 4 = black necrosis.

The tubers used for the tuber slice assay were taken from replicates 1-3 of the pot trials. The tubers from each replicate were placed in a single Petri dish (10 disks), and assessed as a single unit. Each treatment was replicated three times (three Petri dishes with 30 disks in total).

2.3.8 Tuber 2,4-D quantification

The amounts of 2,4-D within tubers at harvest were quantified by selecting 2-3 disease free tubers of a representative size from each treatment within a single replicate. Tuber tissue (10g) was cut into approximately 1cm^2 pieces and stored in cold 80% methanol with butylated hydroxytoluene (BHT) at -20°C. Samples were homogenised with a stick blender with additional 80% methanol with BHT and left overnight at 4°C, after which they were filtered by vacuum filtration through Whatman No.1 filter paper to remove insoluble material. 90ng of 2,4-D internal standard ([$^{13}C_6$] 2,4-D, 100ng/µL in acetone, Catalogue no. XA11940 200AC., Dr. Ehrenstorfer Laboratories, Augsburg, Germany) was added to 10% aliquots of each sample, which were then stored at -20°C. The methanol was removed from the samples in a sample concentrator and then prepared for analysis using Sep Pak (Waters VacRC 500mg C18 cartridges) cartridges. The cartridge was prepared by washing with 100% methanol, then by 0.4% acetic acid. Two 1mL volumes of 0.4% acetic acid were used to transfer the samples from the vial to the Sep Pak cartridge. The samples were then eluted from the cartridge with 3mL of acetonitrile and stored at -20°C. The acetonitrile was removed in a sample concentrator, and 100µL of 0.4% acetic acid was used to transfer the sample to a microcentrifuge tube. The samples were centrifuged at 13,000rpm for 3 minutes to remove any remaining particulate matter, and 70µL was transferred to the final sample tube for analysis. Samples were kept in the dark at room temperature prior to analysis.

UPLC-MS analyses were undertaken on a Waters Acquity H-series UPLC coupled to a Waters Xevo triple quadrupole mass spectrometer. The column was a Waters Acquity UPLC BEH C18 (2.1 x 100mm x 1.7 micron particles), with mobile phases A = 1% acetic acid in water, and B = acetonitrile. The flow rate was 0.3mL/min, using a gradient starting from a 70:30 ratio of solvents A:B, then a linear ramp to 30:70 A:B at 4.5 minutes, and then held for 30 seconds, before immediate re-equilibration to initial conditions for 3 minutes. The column was held at 45C°.

The mass spectrometer was operated in negative ion electrospray mode, using multiple reaction monitoring (MRM) mode. The ion source temperature was 130°C, the desolvation gas was nitrogen at 950L/hr, the cone gas was nitrogen at 50L/hr, the desolvation temperature was 450°C and the capillary voltage was 2.7KV.

MRM transitions for quantitation were:

2,4-D	*m/z* 219 to 161, cone voltage 20V, collision energy 18V
$^{13}C_6$ 2,4-D	*m/z* 225 to m/z 167, cone voltage 20V, collision energy 18V

For confirmation of each analyte the following channels were also monitored:

2,4-D	*m/z* 219 to 125, cone voltage 20V, collision energy 28V
	m/z 221 to 163, cone voltage 20V, collision energy 18V
$^{13}C_6$ 2,4-D	*m/z* 227 to 169, cone voltage 20V, collision energy 18V

Dwell time for all channels was 95ms.

2.3.9 Data analysis

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009). Multivariate Analysis of Variance was used to determine significant effects from, and interactions between treatment factors. Probabilities less than 0.05 were considered to be significant and Fischer's least significant difference (LSD) test was used for comparison of treatment means. Linear Regressions were used to examine relationships between powdery scab and common scab severity.

2.4 Results

2.4.1 Disease control

2.4.1.1 Pot Trials

In pot trial #1 there was no significant effect found from any of the 2,4-D treatments on common scab incidence or severity. Variety was found to have a significant effect on all measures of disease incidence and severity. Desiree had a greater proportion of diseased tubers, and these had significantly greater mean disease cover scores (DCS) and lesion depth scores (LDS) than those in Russet Burbank (Table 2.1).

In pot trial #2 there was a significant effect found from the date of the first spray on DCS, LDS, and the proportion of disease tubers to healthy tubers. The control and 50 DAE treatments were not significantly different in DCS or LDS, and all other treatments resulted in lower DCS and LDS than the control treatment (with the exception of the 20 DAE treatment, which was not significantly different in LDS to the control and 50 DAE treatments). The control and 50 DAE treatments were not significantly different in the proportion of diseased tubers, and all treatments resulted in significantly lower proportions of diseased tubers than the control treatment, and all except the 20 DAE treatment resulted in significantly lower proportion of disease than the 50 DAE treatment. All treatments including the control resulted in low disease severity and incidence in both pot trials. Generally earlier treatments resulted in lower DCS than later and control treatments, and in lower numbers of tubers with visible lesions than later treatments, except for treatments with a first spray date of 40 DAE, which were not significantly different from the earliest treatments. In pot trial #2 there was no effect found from variety for any measures of disease severity, but the proportion of diseased tubers was significantly higher in Russet Burbank than in Desiree, the opposite of the effect found in pot trial #1 (Table 2.2).

Table 2.1 Pot trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on common scab disease severity of tubers that had any visible lesions measured as a) the area of tuber surface covered by scab lesions using the Disease Cover Score (section 2.3.6), b) the derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using the Lesion Depth Score (section 2.3.6) and common scab disease incidence measured as the number of tubers with visible lesions as a proportion of total tubers per pot. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.1084, LSD = 0.2143), LDS ($p < 0.001$, SED = 0.1185, LSD = 0.2343) and proportion of diseased tubers ($p = 0.003$, SED = 2.22, LSD = 4.4). There was no significant effect found from date of first spray or number of sprays, or interactions between factors. Treatments with the same letter in the same variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Frequency	Rate	Date (DAE)	Disease Cover Score (and Percentage Cover (%))			Lesion Depth Score			Proportion Diseased/Pot (%)		
			Russet Burbank	Desiree	Mean	R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean
Single	**200mg/L**	**10**	0.00	0.40 (1.20%)	0.20	0.00	0.60	0.30	0.0	10.7	5.3
		20	0.20 (0.60%)	0.00	0.10	0.40	0.00	0.20	5.0	0.0	2.5
		30	0.40 (1.50%)	0.00	0.20	0.40	0.00	0.20	10.0	0.0	5.0
		40	0.00	0.50 (2.75%)	0.25	0.00	0.50	0.30	0.0	4.0	2.0
		50	0.00	0.40 (1.50%)	0.20	0.00	0.20	0.10	0.0	3.3	1.7
		Control	0.00	1.00 (5.50%)	0.50	0.00	1.00	0.50	0.0	25.0	12.5
Double	**200mg/L**	**10**	0.00	1.10 (4.85%)	0.55	0.20	1.20	0.70	0.0	21.2	10.6
		20	0.00	0.80 (3.00%)	0.40	0.00	0.60	0.30	0.0	10.0	5.0
		30	0.20 (0.60%)	1.00 (5.50%)	0.60	0.40	1.00	0.70	6.7	10.0	8.3
		40	0.40 (1.50%)	0.40 (1.50%)	0.40	0.40	0.20	0.30	4.0	4.0	4.0
		50	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0
		Control	0.30 (0.70%)	0.87 (3.83%)	0.58	0.20	0.60	0.40	10.0	16.0	13.0
Double	**100mg/L**	**10**	0.00	0.50 (1.60%)	0.25	0.00	0.60	0.30	0.0	5.1	2.5
		20	0.00	0.00	0.00	0.00	0.20	0.10	0.0	0.0	0.0
		30	0.30 (0.70%)	0.80 (3.00%)	0.55	0.40	1.00	0.70	9.0	15.0	12.0
		40	0.40 (1.50%)	1.10 (3.70%)	0.75	0.40	1.30	0.90	3.3	21.4	12.4
		50	0.20 (0.60%)	1.10 (4.05%)	0.65	0.20	1.10	0.70	4.0	25.0	14.5
		Control	0.20 (0.60%)	0.60 (2.10%)	0.40	0.20	1.20	0.70	4.0	5.3	4.7
Mean		**10**	0.00	0.67	0.33	0.07	0.80	0.43	0.0	12.3	6.1
		20	0.07	0.27	0.17	0.13	0.27	0.20	1.7	3.3	2.5
		30	0.30	0.60	0.45	0.40	0.67	0.53	8.6	8.3	8.4
		40	0.27	0.67	0.47	0.27	0.67	0.47	2.4	9.8	6.1
		50	0.07	0.50	0.28	0.07	0.43	0.25	1.3	9.4	5.4
		Control	0.17	0.82	0.49	0.13	0.93	0.53	4.7	15.4	10.1
		Mean (Var.)	0.14 a	0.59 b		0.18 a	0.63 b		3.1 a	9.8 b	

Table 2.2 Pot trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on common scab disease severity of tubers that had any visible lesions measured as a) the area of tuber surface covered by scab lesions using DCS (section 2.3.6), b) the derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using LDS (section 2.3.6) and common scab disease incidence measured as the number of tubers with visible lesions as a proportion of total tubers per pot. There was a significant effect found from date of first spray on DCS ($p < 0.001$, SED = 0.1146, LSD = 0.2266), LDS ($p = 0.003$, SED = 0.1448, LSD = 0.2864) and from date of first spray ($p = 0.003$, SED = 4.41, LSD = 8.73) and variety ($p = 0.003$, SED = 2.25, LSD = 5.04) on proportion of diseased tubers. There were no other significant effects on variables, or interactions between factors. Treatments with the same letter in the same column, or row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Disease Cover Score (and Percentage Cover (%))			Lesion Depth Score			% Diseased Tubers/Pot		
			Russet Burbank	Desiree	Mean	R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean
Single	**200mg/L**	**10**	0.15 (0.35%)	0.10 (0.10%)	0.13	0.20	0.20	0.20	13.3	5.0	9.2
		20	0.20 (0.60%)	0.00	0.10	0.60	0.20	0.40	10.0	0.0	5.0
		30	0.20 (0.20%)	0.35 (0.95%)	0.28	0.40	0.60	0.50	15.3	8.2	11.8
		40	0.10 (0.10%)	0.20 (0.60%)	0.15	0.20	0.40	0.30	2.9	5.0	3.9
		50	0.55 (1.55%)	0.80 (2.30%)	0.68	1.00	1.20	1.10	23.3	15.2	19.3
		Control	0.70 (1.50%)	0.75 (2.45%)	0.73	1.10	0.80	0.95	38.0	11.2	24.6
Double	**200mg/L**	**10**	0.10 (0.10%)	0.00	0.05	0.40	0.00	0.20	4.0	0.0	2.0
		20	0.10 (0.10%)	0.50 (1.25%)	0.30	0.40	0.70	0.55	8.0	10.3	9.2
		30	0.10 (0.10%)	0.50 (1.60%)	0.30	0.20	0.40	0.30	6.7	5.3	6.0
		40	0.10 (0.10%)	0.70 (2.20%)	0.40	0.40	0.80	0.60	5.0	8.1	6.5
		50	0.40 (1.20%)	0.30 (0.30%)	0.35	0.40	0.60	0.50	16.7	8.5	12.6
		Control	0.30 (0.70%)	0.55 (1.55%)	0.43	0.40	0.80	0.60	23.3	12.9	18.1
Double	**100mg/L**	**10**	0.20 (0.20%)	0.30 (0.70%)	0.25	0.40	0.80	0.60	14.0	6.9	10.4
		20	0.30 (0.70%)	0.30 (0.30%)	0.30	0.40	1.00	0.70	26.7	10.6	18.7
		30	0.10 (0.10%)	0.10 (0.10%)	0.10	0.40	0.20	0.30	10.0	1.8	5.9
		40	0.00	0.25 (0.80%)	0.13	0.20	0.40	0.30	0.0	6.7	3.3
		50	0.60 (1.00%)	0.30 (0.70%)	0.45	1.00	0.60	0.80	32.5	6.2	19.3
		Control	0.60 (1.80%)	0.60 (1.80%)	0.60	0.60	0.70	0.65	23.0	11.9	17.5
Mean		**10**	0.15	0.13	0.14 a	0.33	0.33	0.33 a	10.4	4.0	7.2 a
		20	0.20	0.27	0.23 a	0.47	0.63	0.55 ab	14.9	7.0	10.9 ab
		30	0.13	0.32	0.23 a	0.33	0.40	0.37 a	10.7	5.1	7.9 a
		40	0.07	0.38	0.23 a	0.27	0.53	0.40 a	2.6	6.6	4.6 a
		50	0.52	0.47	0.49 b	0.80	0.80	0.80 b	24.2	10.0	17.1 bc
		Control	0.53	0.63	0.58 b	0.70	0.77	0.73 b	28.1	12.0	20 c
		Mean (Var.)	0.27	0.37		0.48	0.58		15.1 a	7.4 b	

2.4.1.2 Field Trial #1

In field trial #1 there was a significant interaction found from the date of the first 2,4-D application, the number of applications, and variety on both measures of disease severity (disease incidence was not recorded in the field trials due to the very high proportion of tubers with disease across all treatments; Table 2.3).

In Russet Burbank mean disease cover increased with increasing first spray date for single, double, and triple sprays. Treatments with the same first application date were not significantly different from each other, except those with an initial application at 30 DAE, which had a lower surface coverage in the single spray treatment than the double and triple. All treatments applied with an initial spray date of 30 DAE or earlier resulted in significantly lower DCS than the control treatment, and all those with a first spray date of 40-60 DAE were not significantly different from the control. In the single and double sprays the 30 DAE treatments were not significantly different to the later applications, while in the triple spray treatments the 30DAE initial spray treatment resulted in significantly lower DCS than the 40 DAE initial spray treatment. (Table 2.3)

In Desiree mean disease cover increased with increasing first spray date for single sprays, but this trend was less obvious in the double and triple spray treatments. All treatments resulted in a lower DCS than the control treatment. In the double spray treatments the 10 DAE initial spray was significantly lower than the 50 DAE initial spray, but there were no other significant difference between the treatments. In the triple spray treatments the 30 DAE initial spray was significantly lower than the 40 DAE initial spray treatment, but there were no other significant differences between the treatments. Treatments with the same first spray date of 10, 20 and 50 DAE were not significantly different from each other. The single spray treatment applied at 30 DAE had a significantly higher DCS than the corresponding double spray treatment, and the single spray treatment applied at 40 DAE had a significantly higher DCS than the corresponding triple spray treatment. (Table 2.3)

Lesion Depth Score (LDS) showed a similar pattern as DCS in both varieties. In Russet Burbank all treatments resulted in lower LDS than the control treatment except the 40 DAE and 60 DAE single spray, and the triple spray with an initial

application date of 40 DAE. There was a trend towards increasing LDS with increasing initial spray dates for single, double and triple sprays. All treatments with the same initial spray date were not significantly different from each other, except the 30 DAE single spray treatment, which resulted in significantly lower LDS than the corresponding double and triple spray treatments. In Desiree, all treatments resulted in lower LDS than the control treatment. In the single spray treatments there was a trend towards increasing LDS with increasing spray date, although the 30-60 DAE sprays were not significantly different from each other. In the double spray treatments, the 10 DAE treatment had significantly lower LDS than the 50 DAE initial spray treatment, and all other treatments were not significantly different from each other. In the triple spray treatments, the 30 DAE treatment had a significantly higher LDS than the 40 DAE initial spray treatment, and all other treatments were not significantly different from each other. (Table 2.3)

Table 2.3 Field trial #1 The effect of 2,4-D applied at various times, and as single, double and triple sprays to the foliage of Russet Burbank and Desiree plants, on common scab disease severity of tubers that had any visible lesions measured as a) the area of tuber surface covered by scab lesions using DCS (section 2.3.6), b) the derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using LDS (section 2.3.6). There was a significant interaction between date of first spray, number of sprays and variety on DCS ($p = 0.027$, SED = 0.2286, LSD = 0.4539) and LDS ($p = 0.024$, SED = 0.2193, LSD = 0.4355). Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Disease Cover Score (and Percentage Cover %)							Lesion Depth Score				
			Russet Burbank			Desiree			Mean	Russet Burbank		Desiree		Mean
Single	**100mg/L**	**10**	1.57	a	(5.83%)	2.28	ab	(12.16%)	1.92	1.80	abc	2.49	abc	2.15
		20	1.66	ab	(6.52%)	2.44	abc	(14.78%)	2.05	2.12	bc	2.65	abcd	2.39
		30	2.08	b	(10.81%)	2.79	cde	(19.25%)	2.43	2.16	cd	3.03	def	2.60
		40	2.78	cd	(18.38%)	2.91	de	(20.82%)	2.84	2.95	efg	2.89	ef	2.92
		50	2.79	cd	(19.54%)	3.00	e	(23.11%)	2.89	2.79	ef	2.95	f	2.87
		60	2.95	cd	(21.27%)	3.03	e	(22.85%)	2.99	3.04	fg	3.00	f	3.02
Double	**200mg/L**	**10**	1.48	a	(5.35%)	2.14	a	(10.91%)	1.81	1.70	ab	2.52	a	2.11
		20	1.68	ab	(7.17%)	2.51	abcd	(14.81%)	2.09	1.89	abc	2.78	abcde	2.33
		30	2.55	c	(15.44%)	2.34	ab	(13.54%)	2.45	2.64	ef	2.82	abc	2.73
		40	2.79	cd	(19.00%)	2.50	abcd	(16.34%)	2.64	2.60	de	2.68	abcde	2.64
		50	2.77	cd	(18.38%)	2.66	bcde	(18.00%)	2.71	2.71	ef	2.92	cdef	2.83
Triple	**300mg/L**	**10**	1.35	a	(4.56%)	2.25	ab	(12.16%)	1.80	1.58	a	2.73	abc	2.15
		20	1.78	ab	(8.00%)	2.21	ab	(12.42%)	1.99	2.01	bc	2.71	ab	2.36
		30	2.61	c	(16.68%)	2.61	bcde	(16.26%)	2.61	2.76	ef	2.68	bcdef	2.72
		40	3.21	d	(20.98%)	2.15	a	(12.28%)	2.68	2.94	efg	2.46	a	2.70
	Control		3.20	d	(25.20%)	3.58	f	(32.04%)	3.39	3.27	g	3.32	g	3.30
Mean		**10**	1.47			2.22			1.84	1.69		2.58		2.14
		20	1.71			2.39			2.05	2.01		2.71		2.36
		30	2.41			2.58			2.50	2.52		2.84		2.68
		40	2.93			2.52			2.72	2.83		2.68		2.75
		50	2.78			2.83			2.80	2.75		2.93		2.84
		60	2.95			3.03			2.99	3.04		3.00		3.02
		Control	3.20			3.58			3.39	3.27		3.32		3.30
		Mean (Var.)	2.33			2.59				2.43		2.79		

In both varieties all the 2,4-D sprays resulted in lower powdery scab DCS than the control treatments. In Russet Burbank the 20 DAE application resulted in the lowest powdery scab cover, but was not significantly different from the 10 DAE spray. The 30 DAE spray was not significantly different from either the 10 DAE and 60 DAE spray, and the 40, 50 and 60 DAE sprays resulted in the highest powdery scab DCS, though less than the control, and were not significantly different from each other. In Desire the 10 through 40 DAE applications resulted in the lowest powdery scab DCS and were not significantly different from each other. The 50 and 60 DAE applications resulted in higher powdery scab DCS but were not significantly different from the 20 DAE and 40 DAE applications. In both varieties the control treatment resulted in the highest powdery scab DCS (Table 2.4). There was a moderate positive correlation ($r^2 = 0.49$) between the mean DCS of common scab and powdery scab (Figure 2.2).

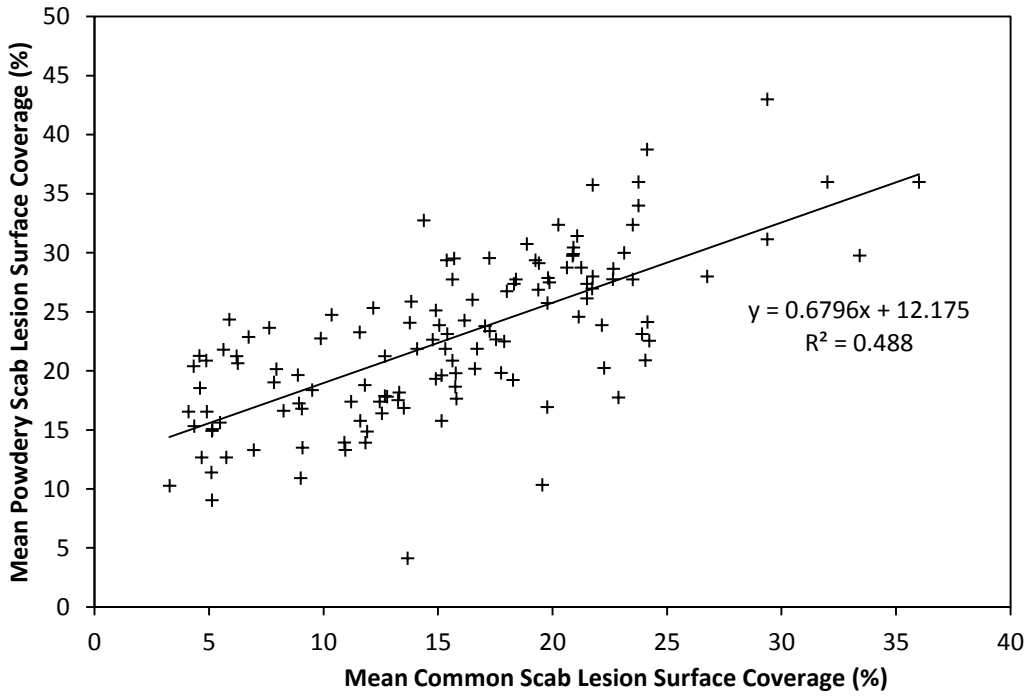

Figure 2.2 Field trial #1 Linear regression between the mean surface coverage of common scab and powdery scab, on tubers harvested from Russet Burbank and Desiree plants treated with 2,4-D at various rates, times, and frequencies. $R^2 = 0.488$.

Table 2.4 Field trial #1 The effect of 2,4-D applied at various times, and as single, double and triple sprays to the foliage of Russet Burbank and Desiree plants, on powdery scab disease severity of tubers that had any visible lesions measured as a) the area of tuber surface covered by scab lesions using DCS (section 2.3.6), and b) the derived percentage cover There was a significant interaction between date of first spray, number of sprays and variety on DCS (p = 0.014, SED = 0.2343, LSD = 0.2654). Treatments with the same letter in the same column are not significantly different at p = 0.05 using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Powdery Scab Disease Cover Score (and Percentage Cover (%)) Russet Burbank			Desiree			Mean
Single	**100mg/L**	**10**	2.79	ab	(20.11%)	2.68	bcde	(17.32%)	2.74
		20	2.78	ab	(18.40%)	3.06	efg	(23.32%)	2.92
		30	2.96	abc	(21.99%)	2.43	ab	(15.07%)	2.70
		40	3.27	cd	(26.77%)	3.24	g	(26.14%)	3.25
		50	3.33	cde	(28.11%)	3.13	efg	(25.72%)	3.23
		60	3.29	cde	(28.11%)	3.08	efg	(23.24%)	3.18
Double	**200mg/L**	**10**	2.70	ab	(17.59%)	2.57	abcd	(15.81%)	2.64
		20	2.67	ab	(17.29%)	3.16	fg	(25.21%)	2.91
		30	3.06	abcd	(23.11%)	2.59	abcd	(17.93%)	2.82
		40	3.34	cde	(28.20%)	3.02	defg	(22.76%)	3.18
		50	3.35	cde	(27.97%)	2.97	cdefg	(22.98%)	3.16
Triple	**300mg/L**	**10**	2.63	a	(16.76%)	2.73	bcdef	(18.61%)	2.68
		20	2.70	ab	(18.26%)	2.53	abc	(16.74%)	2.62
		30	3.11	bcd	(24.08%)	2.81	bcdef	(20.15%)	2.96
		40	3.49	de	(30.22%)	2.18	a	(13.31%)	2.83
	Control		3.74	e	(25.30%)	3.73	h	(34.98%)	3.73
Mean		**10**	2.71			2.66			2.68
		20	2.72			2.92			2.82
		30	3.03			2.61			2.83
		40	3.37			2.81			3.09
		50	3.34			3.05			3.20
		60	3.29			3.08			3.18
		Control	3.74			3.73			3.73
		Mean (Var.)	3.08			2.87			

2.4.1.4 Field Trial #2

There was a significant interaction found between spray rate, number of sprays, and date of first spray on mean disease cover measured using the DCS. For the 25mg/L spray group the control treatment was not significantly different to any treatments except the 10 DAE single spray, which had significantly lower DCS. The 30 and 40 DAE single sprays had significantly higher DCS than the other 25mg/L single sprays. In the 100mg/L spray group, the control treatment had significantly higher DCS than the 5 DAE single spray treatment and the 20 DAE double spray treatment. The 30 and 40 DAE double spray treatments had significantly higher DCS than the 5, 10 and 20 DAE double spray treatments, and the 5 and 10 DAE single spray treatments. The 40 DAE treatment was also significantly higher than the 20 DAE single spray treatment. All other 100mg/L treatments are not significantly different from each other. Treatments with the same first spray date and number of sprays are not significantly different, across spray rates. Desiree had significantly higher DCS than Russet Burbank (Table 2.5).

There was a significant interaction found between spray rate, number of sprays and date of first spray on mean LDS. The control treatment had significantly lower LDS than the 30 DAE single spray 25mg/L treatment, and the 40 DAE double spray 100mg/L treatment. All other treatments were not significantly different to the controls. The 5 and 10 DAE single spray 25mg/L treatments had the lowest LDS of all treatments, which were significantly lower than the 30 and 40 DAE single spray treatments and 5, 10, and 30 DAE double spray treatments in the 25mg/L treatment group, and than the 30 DAE single spray and 5, 30 and 40 DAE double spray treatments in the 100mg/L treatment group. All other treatments were not significantly different from each other. Desiree had significantly greater LDS than Russet Burbank (Table 2.6).

Table 2.5 Field trial #2 The effect of 2,4-D applied at various times, rates and as single and double sprays to the foliage of Russet Burbank and Desiree plants, on common scab disease severity of tubers that had any visible lesions measured as a) the area of tuber surface covered by scab lesions using DCS (section 2.3.6) and b) the derived percentage cover (ANOVA not performed on this data). There was a significant interaction between date of first spray, spray rate and number of sprays ($p = 0.007$, SED = 0.26, LSD = 0.5143) and a significant effect from variety ($p < 0.001$, SED = 0.0721, LSD = 0.1426) on DCS. Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate	Frequency	Date (DAE)	Disease Cover Score (and Percentage Cover (%))					
			Russet Burbank		Desiree		Mean	
25mg/L	**Single**	**5**	1.58	(7.54%)	1.15	(3.61%)	1.36	ab
		10	1.00	(3.18%)	1.70	(6.99%)	1.35	a
		20	1.19	(4.45%)	2.00	(10.17%)	1.60	abcde
		30	1.59	(7.47%)	3.00	(21.43%)	2.30	gh
		40	1.82	(9.31%)	2.45	(15.22%)	2.14	fgh
		50	1.20	(4.30%)	1.86	(9.53%)	1.48	abc
	Double	**5**	1.56	(7.12%)	2.70	(18.25%)	2.13	fgh
		10	1.47	(6.44%)	2.58	(19.17%)	2.02	defgh
		20	1.45	(6.16%)	2.23	(11.59%)	1.84	abcdefg
		30	1.52	(6.09%)	2.65	(16.68%)	2.08	efgh
		40	1.67	(7.68%)	2.62	(15.95%)	2.08	efgh
	Control		1.17	(4.18%)	2.58	(16.05%)	1.87	bcdefg
100mg/L	**Single**	**5**	1.34	(5.43%)	1.77	(8.63%)	1.56	abcd
		10	0.82	(2.05%)	2.67	(16.83%)	1.74	abcdef
		20	1.50	(8.34%)	2.15	(11.96%)	1.82	abcdefg
		30	1.40	(5.55%)	2.46	(16.65%)	1.93	cdefgh
		40	1.54	(6.41%)	2.44	(13.06%)	1.93	cdefgh
		50	1.22	(4.56%)	2.67	(17.08%)	1.94	cdefgh
	Double	**5**	1.42	(7.00%)	2.30	(12.63%)	1.71	abcdef
		10	1.05	(4.08%)	2.25	(12.42%)	1.65	abcdef
		20	1.05	(3.37%)	1.94	(8.14%)	1.50	abc
		30	1.67	(8.71%)	3.00	(22.95%)	2.34	gh
		40	1.70	(7.40%)	3.33	(26.67%)	2.40	h
	Control		1.67	(7.37%)	2.62	(17.43%)	2.15	fgh
Mean		**5**	1.47		1.93		1.69	
		10	1.08		2.30		1.69	
		20	1.31		2.08		1.69	
		30	1.56		2.80		2.18	
		40	1.68		2.69		2.14	
		50	1.21		2.32		1.73	
		Control	1.42		2.60		2.01	
		Mean (Var.)	1.40 a		2.38 b			

Table 2.6 Field trial #2 The effect of 2,4-D applied at various times, rates and as single and double sprays to the foliage of Russet Burbank and Desiree plants, on common scab disease severity of tubers that had any visible lesions measured as the depth of lesions measured using LDS (section 2.3.6). There was a significant interaction between spray rate, number of sprays and date of first spray ($p = 0.011$, SED = 0.1955, LSD = 0.3868) and a significant effect from variety ($p < 0.001$, SED = 0.1073, LSD = 0.1517). Treatments with the same letter in the same column or row are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate	Frequency	Date (DAE)	Lesion Depth Score			
			Russet Burbank	Desiree	Mean	
25mg/L	**Single**	**5**	1.02	1.06	1.04	a
		10	1.09	1.06	1.07	a
		20	1.04	1.29	1.17	abc
		30	1.29	2.25	1.77	ef
		40	1.38	1.64	1.51	cde
		50	1.08	1.50	1.26	abcd
	Double	**5**	1.36	1.80	1.58	de
		10	1.13	1.80	1.47	cde
		20	1.18	1.44	1.31	abcd
		30	1.21	1.69	1.45	bcde
		40	1.20	1.67	1.40	abcde
	Control		1.11	1.58	1.34	abcd
100mg/L	**Single**	**5**	1.21	1.54	1.37	abcd
		10	1.06	1.63	1.34	abcd
		20	1.15	1.29	1.22	abcd
		30	1.04	1.91	1.48	cde
		40	1.15	1.67	1.37	abcd
		50	1.10	1.67	1.38	abcd
	Double	**5**	1.27	2.05	1.53	cde
		10	1.17	1.68	1.43	abcde
		20	1.11	1.44	1.28	abcd
		30	1.23	1.93	1.58	de
		40	1.32	2.83	1.97	f
	Control		1.22	1.42	1.32	abcd
Mean		**5**	1.21	1.55	1.37	
		10	1.11	1.54	1.33	
		20	1.12	1.36	1.24	
		30	1.20	1.95	1.57	
		40	1.26	1.93	1.56	
		50	1.09	1.60	1.33	
		Control	1.16	1.50	1.33	
		Mean (Var.)	1.17 a	1.64 b		

2.4.3 Agronomic effects

2.4.3.1 Pot Trials

In pot trial #1 there was a significant effect found from the date of first treatment on the total mass of tubers per pot. The control and treatments with a first application date of 40 DAE resulted in the highest total tuber mass, but were not significantly different from 10 DAE treatments, Treatments with a first application date of 20, 30 and 50 DAE had tuber masses significantly lower than the control treatment. Treatments with a first application date of 50 DAE were not significantly different from any of the 2,4-D treatments except the treatment applied at 40 DAE, which it was significantly lower than. The treatments with first spray dates of 20 and 30 DAE had the lowest tuber masses. There was no significant effect found from first spray date on mean mass per tuber or number of tubers per pot, and there was no significant effect found from variety or spray rate and frequency on any of the agronomic features recorded (Table 2.7).

In pot trial #2 there was a significant interaction between first spray date, and rate and frequency of application, on the total tuber mass (Table 2.8). In the single application treatment category the control treatment had a significantly higher total tuber mass then all other treatments, except the 50 DAE treatment. This control treatment was also significantly higher than the other two control treatments. There was a general trend for the tuber masses to increase with increasing first application date in the single and double higher rate treatment categories. In the single spray rate category the 10 DAE treatment was not significantly different to the 30 or 40DAE treatment, and the 20 DAE treatment was not significantly different to any treatments except the control, which it was lower than, and the 10 DAE treatment, which it was higher than. In the double higher rate category the 10 DAE treatment was significantly lower than the 30 and 50 DAE treatments and the control treatments, but not significantly different to all other treatments. There was a similar trend in the double lower rate treatment category, but there was no significant difference between application date treatments within this category. All treatments across all categories, with the same first spray date were not significantly different from each other, with the exception of the 30 DAE initial spray date, which had significantly higher total

Table 2.7 Pot trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on total tuber mass per pot, mean mass per tuber and number of tubers per pot. There was a significant effect found from the date of the first spray on total tuber mass ($p < 0.001$, SED = 2.3577, LSD = 4.6612). There was no significant effect found from number of sprays or variety on total tuber mass. There was no significant effect found from date of first spray, number of sprays, or variety on mean tuber mass or number of tubers per pot. There were no significant interactions between factors. Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Total Tuber Mass/Pot (g)			Mean Tuber Mass (g)			Number of Tubers/Pot		
			Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean
Single	**200mg/L**	**10**	25.57	28.15	36.02	8.97	7.16	8.07	5.2	5.2	5.2
		20	27.71	23.08	23.41	4.70	5.62	5.16	6.8	4.0	5.4
		30	22.85	21.15	27.70	7.01	5.69	6.35	5.0	4.8	4.9
		40	33.72	30.18	28.08	4.76	6.16	5.46	4.6	7.2	5.9
		50	21.15	28.14	25.20	6.53	5.37	5.95	4.2	5.8	5.0
		Control	31.96	36.67	33.16	7.01	9.17	8.09	4.6	5.4	5.0
Double	**200mg/L**	**10**	28.44	19.23	23.83	6.07	3.9	4.98	6.2	5.6	5.9
		20	20.55	23.48	22.09	5.64	5.28	5.46	4.4	5.2	4.8
		30	18.68	19.23	18.96	5.01	4.13	4.57	4.0	5.0	4.5
		40	29.95	29.31	29.63	5.76	8.33	7.04	6.0	4.0	5.0
		50	22.36	24.51	23.43	5.42	4.94	5.18	4.8	5.4	5.1
		Control	27.43	37.69	32.56	3.47	7.33	5.40	8.2	5.4	6.8
Double	**100mg/L**	**10**	38.45	33.60	26.86	4.22	5.02	4.62	6.4	6.2	6.3
		20	25.27	21.54	25.40	5.74	3.66	4.70	5.6	6.6	6.1
		30	27.50	27.90	22.00	4.97	8.7	6.83	5.0	4.8	4.9
		40	20.80	35.36	31.95	8.85	6.36	7.60	4.4	4.8	4.6
		50	27.16	23.24	24.64	5.77	5.39	5.58	4.6	6.0	5.3
		Control	28.97	37.34	34.32	7.16	8.88	8.02	4.8	5.4	5.1
Mean		**10**	30.82	26.99	28.91 bc	6.42	5.36	5.89	5.9	5.7	5.8
		20	24.51	22.70	23.61 a	5.36	4.85	5.11	5.6	5.3	5.4
		30	23.01	22.76	22.89 a	5.66	6.17	5.92	4.7	4.9	4.8
		40	28.16	31.62	29.89 c	6.45	6.95	6.70	5.0	5.3	5.2
		50	23.56	25.29	24.43 ab	5.91	5.23	5.57	4.5	5.7	5.1
		Control	29.46	37.24	33.35 c	5.88	8.46	7.17	5.9	5.4	5.6
		Mean (Var.)	26.59	27.77		5.95	6.17		5.3	5.4	

Table 2.8 Pot trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on total tuber mass per pot, mean mass per tuber and number of tubers per pot. There was a significant effect found from variety on total tuber mass per pot ($p < 0.033$, SED = 1.549, LSD = 3.062), mean mass per tuber ($p < 0.001$, SED = 0.724, LSD = 1.432) and number of tubers ($p < 0.001$, SED = 0.3, LSD = 0.593). There was a significant interaction found between date of first spray and number of sprays on total tuber mass per pot ($p = 0.013$, SED = 4.646, LSD = 9.185). There was no significant effect found from number of sprays or variety on total tuber mass. There was no significant effect found from date of first spray or number of sprays, or interactions between factors, on mean mass per tuber or number of tubers. Treatments with the same letter in the same column, or row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Total Tuber Mass/Pot (g)				Mean Tuber Mass (g)			Number of Tubers/Pot		
			R.Burbank	Desiree	Mean		R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean
Single	**200mg/L**	**10**	23.48	29.96	26.72	a	6.86	7.60	7.23	3.8	5.8	4.8
		20	34.08	40.70	37.39	bcd	9.62	7.30	8.46	4.2	6.6	5.4
		30	29.47	30.74	30.11	ab	5.60	5.56	5.58	5.6	6.0	5.8
		40	36.84	29.43	33.13	abc	9.07	5.94	7.51	4.2	5.4	4.8
		50	41.33	47.65	44.49	de	13.23	7.30	10.26	4.0	6.8	5.4
		Control	44.83	55.10	49.97	e	12.65	8.36	10.50	3.8	7.2	5.5
Double	**200mg/L**	**10**	25.22	28.76	26.99	a	7.52	3.67	5.60	3.6	7.2	5.4
		20	29.21	29.02	29.11	ab	12.36	6.58	9.47	3.2	6.0	4.6
		30	41.57	39.39	40.48	cd	14.87	6.47	10.67	3.2	7.6	5.4
		40	28.31	35.81	32.06	abc	11.03	5.60	8.31	3.4	6.6	5.0
		50	33.46	41.51	37.49	bcd	12.36	6.36	9.36	3.0	7.8	5.4
		Control	38.72	33.64	36.18	bcd	15.85	8.10	11.98	2.8	6.0	4.4
Double	**100mg/L**	**10**	29.31	39.06	34.19	abc	9.97	6.62	8.30	3.4	5.8	4.6
		20	32.23	30.86	31.55	abc	10.79	5.60	8.20	3.8	6.4	5.1
		30	36.85	38.72	37.78	bcd	14.21	6.81	10.51	3.0	6.8	4.9
		40	32.96	39.93	36.45	bcd	12.44	6.85	9.64	3.6	5.8	4.7
		50	39.08	36.38	37.73	bcd	10.16	5.26	7.71	4.4	7.2	5.8
		Control	32.45	42.81	37.63	bcd	9.64	6.35	7.99	3.8	7.4	5.6
Mean		**10**	26.00	32.59	29.30		8.12	5.97	7.04	3.6	6.3	4.9
		20	31.84	33.53	32.68		10.92	6.50	8.71	3.7	6.3	5.0
		30	35.96	36.28	36.12		11.56	6.28	8.92	3.9	6.8	5.4
		40	32.7	35.06	33.88		10.85	6.13	8.49	3.7	5.9	4.8
		50	37.96	41.84	39.90		11.92	6.31	9.11	3.8	7.3	5.5
		Control	38.67	43.85	41.26		12.71	7.60	10.16	3.5	6.9	5.2
		Mean (Var.)	33.86 a	37.19 b			11.01 a	6.46 b		3.7 a	6.6 b	

tuber masses in the double, higher spray rate treatment than the other two spray rate and frequency treatments (Table 2.8).

Variety was found to have an effect on all three agronomic features recorded, with Desiree having a higher total tuber mass and number of tubers per pot, while Russet Burbank had a higher mean tuber mass. There was no significant effect found from first spray date or application rate and frequency on the other two agronomic features. (Table 2.8)

2.4.3.2 Field Trials

In field trial #1 there were no significant effects found from any factors on total tuber mass. There was a significant effect found from variety on mean mass per tuber and number of tubers. Russet Burbank had significantly greater number of tubers than Desiree, but Desiree had a significantly higher mean mass per tuber than Russet Burbank (Table 2.9).

In field trial #2 there was a significant interactive effect from spray rate, number of sprays, and date of first spray on mean total mass. However, there is no trend apparent. In the 25mg/L spray group, none of the treatments were significantly different from the control. The 5 DAE single spray had a significantly lower mean total mass than the 40 DAE single spray and 5, 10 and 30 DAE double sprays. In the 100mg/L spray group, the 30 DAE single and double sprays had significantly higher mean total masses than the control treatments, but were not significantly different to the 5 and 20 DAE single sprays, and the 5, 10 and 40 DAE double sprays. There was a significant effect found from date of first spray on mean tuber number. No treatments were significantly different from the control. The 50 DAE treatment had significantly fewer tubers than the 5, 10 and 30 DAE treatments. All other treatments were not significantly different from each other. There was a significant effect found from variety on mean total mass, mean mass per tuber, and number of tubers. Russet Burbank had a significantly greater total tuber mass and number of tubers than Desiree, but Desiree had significantly higher mean mass per tuber than Russet Burbank (Table 2.10).

Table 2.9 Field trial #1 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on total tuber mass, mean mass per tuber and number of tubers. There was a significant effect found from variety on mean tuber mass ($p < 0.001$, SED = 4.92, LSD = 9.76) and number of tubers ($p < 0.001$, SED = 1.684, LSD = 3.345). There were no significant effects found from number of sprays, or date of first spray on mean tuber mass, or interactions between factors, on number of tubers. There was no significant effect found from any factors on total tuber mass. Treatments with the same letter in the same row and factor are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Mean Total Mass (g)			Mean Mass per Tuber (g)			Number of Tubers		
			R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean
Single	**100mg/L**	**10**	3134	4152	3643	93.7	145.0	119.3	33.5	28.5	31.0
		20	3419	3148	3283	107.5	172.7	140.1	33.3	18.8	26.0
		30	3854	3131	3492	111.7	166.0	138.8	35.3	19.0	27.1
		40	2956	2694	2825	91.0	135.9	113.4	32.3	18.5	25.3
		50	3131	3506	3319	125.3	175.2	150.3	25.5	20.3	22.9
		60	3192	3718	3455	102.7	176.5	139.6	30.0	21.3	25.6
Double	**200mg/L**	**10**	3161	3250	3206	70.4	153.8	112.1	44.8	20.8	32.8
		20	3285	3656	3471	97.0	148.0	122.5	34.0	25.3	29.6
		30	3675	2508	3091	112.7	159.1	135.9	34.0	15.5	24.8
		40	4081	3734	3908	115.1	178.0	146.5	35.3	21.5	28.8
		50	3312	2990	3151	111.4	156.6	134.0	33.3	20.0	26.6
Triple	**300mg/L**	**10**	3029	3902	3466	70.7	144.2	107.4	42.8	27.3	35.0
		20	3775	3841	3808	104.9	163.1	134.0	35.8	23.5	29.6
		30	2610	2952	2781	107.1	158.6	132.8	24.0	19.0	21.5
		40	2891	3188	3039	80.6	170.0	125.3	35.8	18.8	27.3
	Control		2332	3761	3047	111.1	198.5	154.8	22.8	19.5	21.1
Mean		**10**	3108	3768	3438	78.2	147.6	112.9	40.3	25.5	32.9
		20	3493	3548	3521	103.1	161.3	132.2	34.3	22.5	28.4
		30	3380	2864	3122	110.5	161.2	135.8	31.1	17.8	24.5
		40	3310	3205	3257	95.6	161.3	128.4	34.4	19.6	27.0
		50	3222	3248	3235	118.3	165.9	142.1	29.4	20.1	24.8
		60	3192	3718	3455	102.7	176.5	139.6	30.0	21.3	25.6
		Control	2332	3761	3047	111.1	198.5	154.8	22.8	19.5	21.1
		Mean (Var.)	3240	3383		100.8 a	162.6 b		33.3 a	21.1 b	

Table 2.10 Field trial #2 The effect of 2,4-D applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants, on total tuber mass, mean mass per tuber and number of tubers. There was a significant effect found from variety on total tuber mass ($p < 0.001$, SED = 48.7, LSD = 96.4) and mean tuber mass ($p < 0.001$, SED = 2.68, LSD = 5.31). There was a significant effect found from variety ($p < 0.001$, SED = 0.809, LSD = 1.599) and date of first spray ($p < 0.001$, SED = 1.786, LSD = 3.53) on tuber number. There was a significant interaction between spray rate, number of sprays and date of first spray on total tuber mass ($p = 0.027$, SED = 175.6, LSD = 347.4) Treatments with the same letter in the same row and factor are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate	Frequency	Rate	Date (DAE)	Mean Total Mass (g)				Mean Mass per Tuber (g)			Number		
				R.Burbank	Desiree	Mean		R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean
25mg/L	**Single**	**25mg/L**	**5**	151.2	75.0	113.1	a	13.7	23.8	18.7	8.3	3.0	5.6
			10	291.2	178.8	235.0	abc	26.8	23.4	25.1	10.8	7.8	9.3
			20	327.5	70.0	198.8	ab	26.4	32.5	29.5	10.5	2.0	6.3
			30	455.0	300.0	377.5	abcdefg	26.3	52.3	39.3	13.5	5.5	9.5
			40	632.5	292.5	462.5	bcdefg	29.4	27.2	28.3	16.8	7.5	12.1
			50	247.5	163.3	211.4	abc	24.0	42.8	32.1	8.0	3.0	5.5
	Double	**50mg/L**	**5**	677.5	497.5	587.5	defg	33.7	44.2	39.0	14.5	11.5	13.0
			10	737.5	252.5	495.0	bcdefg	44.3	37.5	40.9	13.8	6.0	9.9
			20	320.0	460.0	390.0	abcdefg	24.7	26.4	25.6	8.5	7.8	8.1
			30	822.5	442.5	632.5	fg	38.6	53.0	45.8	15.0	5.8	10.4
			40	358.8	110.0	252.1	abcde	29.6	24.1	27.2	11.3	4.0	7.6
		Control		327.5	311.2	319.4	abcdef	31.6	30.8	31.2	8.3	6.5	7.4
100mg/L	**Single**	**100mg/L**	**5**	656.2	536.2	596.3	efg	37.1	38.7	37.9	15.0	10.5	12.8
			10	308.8	178.8	243.8	abcd	20.8	35.6	28.2	13.5	4.8	9.1
			20	586.2	237.5	411.9	abcdefg	41.5	27.8	34.7	12.0	5.8	8.9
			30	716.7	713.3	715.0	g	34.7	38.2	36.5	12.5	12.5	12.5
			40	408.3	91.7	250.0	abcde	33.5	29.4	31.5	10.0	2.8	6.4
			50	280.0	311.2	295.6	abcdef	37.7	74.2	55.9	6.8	4.0	5.4
	Double	**200mg/L**	**5**	672.5	317.5	554.2	cdefg	27.5	50.1	35.0	20.3	3.3	11.8
			10	465.0	328.8	396.9	abcdefg	29.7	32.4	31.1	15.5	10.0	12.8
			20	271.7	91.7	181.7	ab	26.2	21.4	23.8	7.0	2.5	4.8
			30	625.0	761.2	693.1	g	29.2	41.0	35.1	16.0	12.5	14.3
			40	626.2	140.0	417.9	abcdefg	40.3	72.5	54.1	12.3	1.3	6.8
		Control		432.5	206.2	319.4	abcdef	28.5	36.2	32.4	12.5	6.0	9.3

Spray Rate	Frequency	Rate	Date (DAE)	Mean Total Mass (g)			Mean Mass per Tuber (g)			Number			
				R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean	R.Burbank	Desiree	Mean	
Mean			**5**	539.4	362.1	456.7	28.0	37.7	32.5	14.5	7.1	10.8	c
			10	450.6	234.7	342.7	30.4	32.2	31.3	13.4	7.1	10.3	bc
			20	387.9	206.1	297.0	30.3	27.5	28.9	9.5	4.5	7.0	ab
			30	650.7	543.7	597.2	32.0	46.7	39.4	14.3	9.1	11.7	c
			40	513.0	168.8	353.2	33.2	37.4	35.2	12.6	3.9	8.2	abc
			50	263.8	247.9	256.3	30.8	60.7	44.8	7.4	3.5	5.4	a
			Control	380.0	258.8	319.4	30.1	33.5	31.8	10.4	6.3	8.3	abc
			Mean (Var.)	476.9 a	297.3 b		30.7 a	38.0 b		12.2 a	6.1 b		

2.4.4 Toxin tolerance

2.4.4.1 Pot Trials

In pot trial #1, while there was a significant interactive effect from variety, timing of sprays and number of sprays on tuber toxin tolerance after harvest, there is no particular trend apparent (Table 2.11).

In pot trial #2 there was no significant effect from treatment apparent in Desiree. In Russet Burbank all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control treatment, except the earliest treatment applied at 10 DAE. The 10, 20, 40 and 50 DAE treatments were not significantly different from each other. The 30 DAE treatment resulted in a necrosis score significantly lower (or significantly higher toxin tolerance) than all other treatments (Table 2.11).

Table 2.11 The effect of 2,4-D foliar sprays on mean necrosis levels from the treatment of tuber slices with thaxtomin A. 2,4-D foliar sprays were applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants in two pot trials. Necrosis was rated using the scale described in section 2.3.7. There was a significant interaction between first spray date, number of sprays and variety in pot trial #1 ($p < 0.001$, SED = 0.1273, LSD = 0.2498) and pot trial #2 ($p < 0.001$, SED = 0.1074, LSD = 0.2107). Treatments with the same letter in the same column are not significantly different at $p = 0.05$ using Fischer's LSD test.

Frequency	Rate	Date (DAE)	Trial #1			Trial #2		
			Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean
Single	**200mg/L**	**10**	2.042 cd	1.625 a	1.833	2.219 j	1.427 abc	1.823
		20	2.062 cd	1.875 bcde	1.969	1.667 ef	1.594 bcdef	1.630
		30	1.729 ab	1.854 abcd	1.792	1.188 a	1.688 defg	1.438
		40	2.646 g	2.083 def	2.365	2.000 hi	1.260 a	1.630
		50	2.417 fg	1.771 abc	2.094	1.792 fgh	1.521 bcd	1.656
		Control	1.979 cd	2.063 def	2.021	2.167 ij	1.688 defg	1.927
Double	**200mg/L**	**10**	1.688 ab	1.708 abc	1.698	1.594 def	1.385 ab	1.490
		20	2.354 ef	1.875 bcde	2.115	1.542 cde	1.865 g	1.703
		30	2.062 cd	1.875 bcde	1.969	1.448 bcd	1.896 g	1.672
		40	1.521 a	1.812 abc	1.667	1.250 ab	1.625 cdef	1.438
		50	2.062 cd	2.063 def	2.062	1.938 gh	1.573 bcde	1.755
		Control	2.167 de	2.250 f	2.208	1.927 gh	1.854 g	1.891
Double	**100mg/L**	**10**	1.875 bc	1.667 ab	1.771	1.688 ef	1.760 efg	1.724
		20	2.167 de	1.729 abc	1.948	1.917 gh	1.708 defg	1.813
		30	2.521 fg	1.917 cde	2.219	1.354 abc	1.604 cdef	1.479
		40	1.979 cd	1.938 cde	1.958	1.729 efg	1.802 fg	1.766
		50	2.042 cd	1.771 abc	1.906	1.521 cde	1.635 cdef	1.578
		Control	1.979 cd	2.104 ef	2.042	2.208 ij	1.708 defg	1.958
Mean		**10**	1.868	1.667	1.767	1.833	1.524	1.679
		20	2.194	1.826	2.010	1.708	1.722	1.715
		30	2.104	1.882	1.993	1.330	1.729	1.530
		40	2.049	1.944	1.997	1.660	1.563	1.611
		50	2.174	1.868	2.021	1.750	1.576	1.663
		Control	2.042	2.139	2.090	2.101	1.750	1.925

2.4.4.2 Field Trials

Toxin tolerance was only determined for tubers harvested from field trial #2 (Table 2.12). There was a significant interactive effect found on toxin tolerance between date of first application, application rate, and variety. In Russet Burbank, the control treatment had significantly higher necrosis scores than all 100mg/L treatments, and all 25mg/L treatments except treatments with a first spray date of 20 DAE. Of the 100mg/L treatments, all the scores were not significantly different from each other, except the 10 and 20DAE treatments, which had significantly higher necrosis scores than all other treatments, except the 30 DAE treatment, which was not significantly different to any 100mg/L treatment. Of the 25mg/L treatments, the 5, 10 and 20 DAE treatments were not significantly different to each other, and the 5 and 20 DAE treatments had significantly higher necrosis scores than all other treatments. All other treatments were not significantly different to each other.

In Desiree, the control treatment had a higher necrosis score than all 100mg/L treatments except those with a first spray date of 10 and 20 DAE, and all 25mg/L treatments, except those with a first spray date of 10 DAE. Of the 25mg/L 10 DAE treatment also had higher necrosis scores than all treatments except the 20 DAE treatment, and all other 25mg/L treatments were not significantly different from each other. Of the 100mg/L treatments, the 10 and 20 DAE treatments were not significantly different from each other, and both had significantly higher necrosis scores than all the other treatments, which were not significantly different from each other. (Table 2.12)

Table 2.12 Field trial #2 The effect of 2,4-D foliar sprays on mean necrosis levels from the treatment of tuber slices with thaxtomin A. 2,4-D foliar sprays were applied at various times, rates and frequencies to the foliage of Russet Burbank and Desiree plants. Necrosis was rated using the scale described in section 2.3.7. There was a significant interaction between spray rate, first spray date and variety (p = 0.045, SED = 0.1196, LSD = 0.2352). Treatments with the same letter in the same column are not significantly different at p = 0.05 using Fischer's LSD test.

Number of Sprays	Spray Rate	Date of First Spray (DAE)	Mean Necrosis Score				
			Russet Burbank		Desiree		Mean
Single	**25**	**5**	1.903		1.646		1.775
		10	1.451		1.708		1.580
		20	1.660		1.438		1.549
		30	1.368		1.479		1.424
		40	1.632		1.479		1.556
		50	1.542		1.299		1.421
	100	**5**	1.236		1.444		1.340
		10	1.562		1.632		1.597
		20	1.528		1.806		1.667
		30	1.333		1.146		1.240
		40	1.285		1.562		1.424
		50	1.306		1.222		1.264
	Control		2.007		1.847		1.927
Double	**25**	**5**	1.479		1.340		1.410
		10	1.514		1.715		1.615
		20	1.792		1.583		1.688
		30	1.396		1.347		1.372
		40	1.472		1.458		1.465
	100	**5**	1.347		1.177		1.262
		10	1.521		1.625		1.573
		20	1.493		1.469		1.481
		30	1.458		1.201		1.330
		40	1.264		1.229		1.247
	Control		1.854		1.708		1.781
Mean	**25**	**5**	1.691	b	1.462	cde	1.577
		10	1.483	bcde	1.712	ab	1.598
		20	1.713	ab	1.510	bcde	1.612
		30	1.382	cde	1.413	def	1.398
		40	1.552	bc	1.471	cde	1.512
		50	1.542	bc	1.299	ef	1.421
	100	**5**	1.292	de	1.337	ef	1.315
		10	1.542	bc	1.628	abcd	1.585
		20	1.510	bcd	1.671	abc	1.591
		30	1.396	cde	1.179	f	1.288
		40	1.274	e	1.363	ef	1.319
		50	1.306	de	1.222	f	1.264
	Control		1.931	a	1.778	a	1.855

2.4.5 2,4-D Quantification

2.4.5.1 Field trial #2

Russet Burbank had a significantly greater amount of 2,4-D in tubers at harvest than Desiree. In both varieties there was a trend towards increasing levels of 2,4-D in tubers at harvest with later application dates (Figure 2.3). For most application dates the 100mg/L application rate treatment had higher levels of 2,4-D in the tuber at harvest than the 25mg/L treatment, except the 40 DAE treatment in Russet Burbank and the 10 and 50 DAE treatments in Desiree. The sample taken from Russet Burbank treated at 50 DAE with 100mg/L was the only sample to have greater than Australian maximum residue level (MRL) of 100ng/g of tuber fresh weight (Commonwealth of Australia 2011).

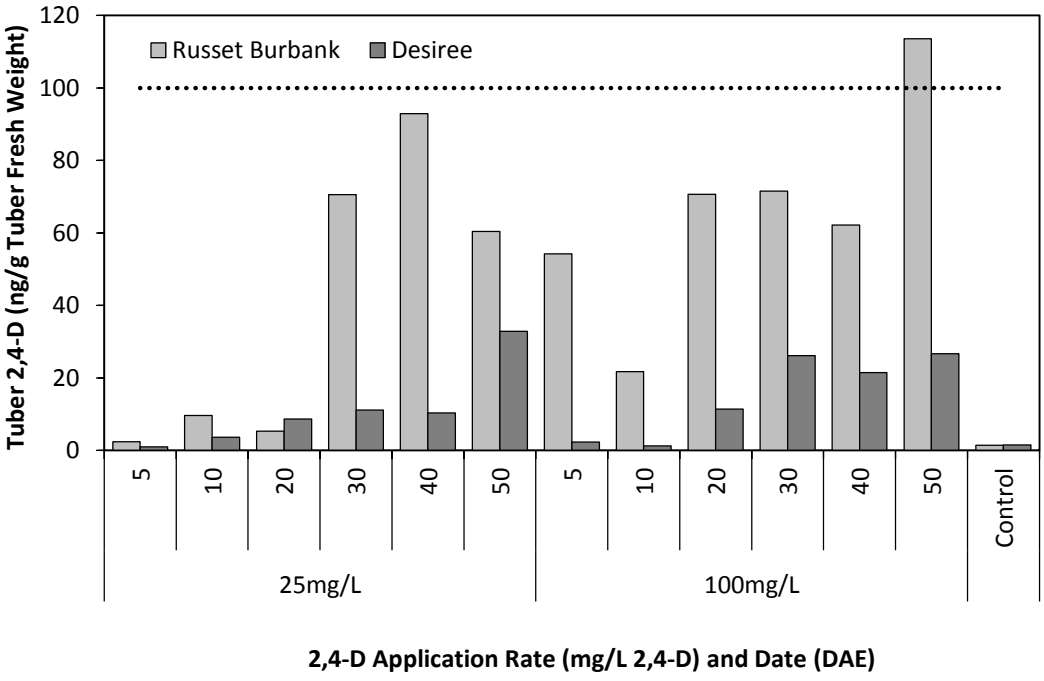

Figure 2.3 Field trial #2 The quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at various times and rates to the foliage of Russet Burbank and Desiree plants. Dotted line indicates the maximum residue limit (MRL) for 2,4-D in potatoes at harvest.

For treatments applied at 20 DAE, the samples taken from Desiree tubers had similar levels of 2,4-D at harvest for both applications rates and for single and double applications. The sample taken from Russet Burbank tubers had similar levels to the Desiree samples for the 25mg/L single treatment, and much greater levels for the other treatments. The 100mg/L single and 25mg/L double treatments were similar, and the 100mg/L double treatment was slightly higher. All treatments resulted in levels at harvest below the MRL. These data lack replication and as such have only been analysed statistically for varietal differences (Figure 2.4).

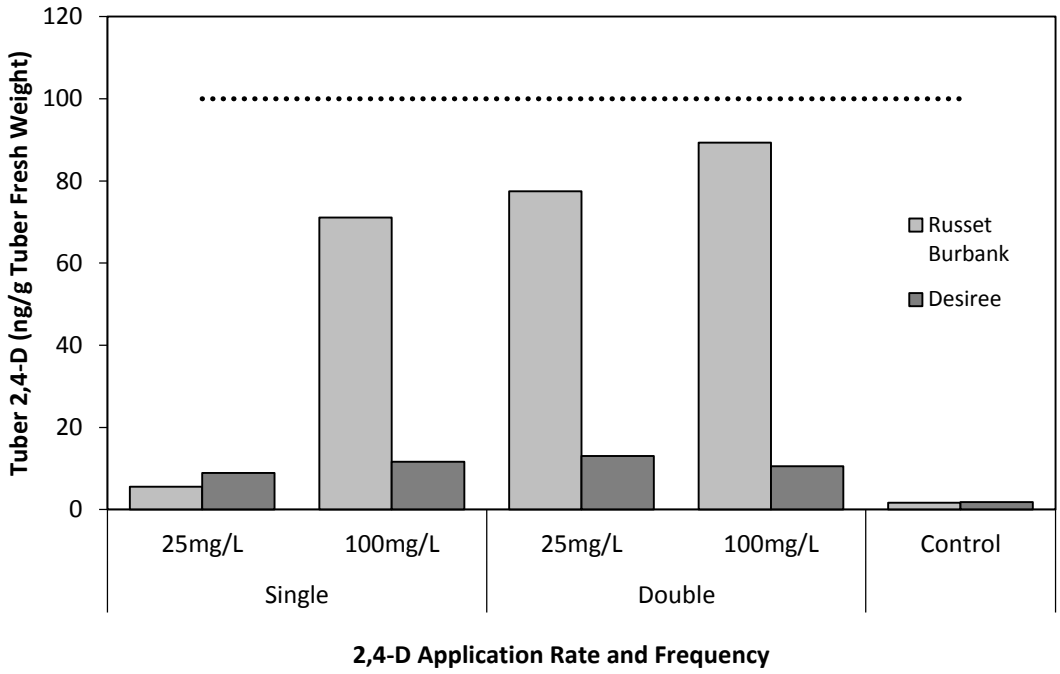

Figure 2.4 Field trial #2 The quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at 20DAE, at a low and high rate and as single and double sprays to the foliage of Russet Burbank and Desiree plants. Dotted line indicates the maximum residue limit (MRL) for 2,4-D in potatoes at harvest.

2.5 Discussion

2.5.1 The earliest treatments prior to tuber initiation provide greatest control

A discrete window of susceptibility of potato tubers to infection with the causal agents of common scab occurs during the rapid growth phase of tuber development. Tuber internodes are susceptible to infection by *S. scabies* after lenticels have been formed from stomata but have not yet completely suberised (Adams 1975). Lapwood & Adams (1973) determined that only when internodes are in the 3rd and 4th position from the apical bud are their lenticels in this susceptible phase. Internodes are susceptible to infection for around 10 days, beginning 1-2.5 weeks after formation (Adams & Lapwood 1978), and individual lenticels remain susceptible for around one week under normal tuber growth rates (Adams 1975).

More recent research has found that earlier formed lenticels are more susceptible to infection than those formed later (Khatri *et al.* 2011), suggesting that tubers will be most susceptible to infection at the beginning of the infection window, making it the most crucial period for protecting tubers against the disease. Khatri *et al*. (2011) observed that inoculating tubers 14 days after tuber initiation resulted in 68% of tubers becoming infected, while only 4% of tubers were infected as a result of inoculations 8 weeks after tuber initiation. That the majority of infection appears to occur at the beginning of the infection window, and that the infection window opens soon after tubers have initiated, suggests that controlling early infection around the time of tuber initiation will be important in controlling subsequent disease. This corresponds with both previous research (Tegg *et al.* 2008), in which the timing of the 2,4-D treatments appeared to be a critical factor in the efficacy of this control method, and the results of this study, in which later treatments were found to be less effective in controlling disease than those applied before or at the commencement of the initiation of the majority of tubers within the trials.

Tegg *et al.* (2008) found that treatments applied well after tuber initiation gave little disease control when compared to treatments with a targeted application of less than 2 weeks after first evidence of tuber initiation. However, this observation had only been made through comparisons between discrete trials, rather than through the comparison of differently timed treatments. McIntosh *et al*. (1981) found that earlier

applications (at 21 DAE) had increased efficacy but also increased the phytotoxic effects, and this led to them not recommending 2,4-D as a practical control method. This earlier application date was similar to the timing adopted by Tegg *et al*. (2008), but may still be after the beginning of the infection window, which changes depending on a variety of physiological and environmental factors. The current study confirmed the observations of an increased efficacy of earlier treatments found by McIntosh *et al*. (1981) and the importance of timing that were made by Tegg *et al*. (2008). It also provides further evidence that treatment with 2,4-D during the infection window suppresses common scab (McIntosh *et al.* 1981; 1982; Tegg 2006; Tegg *et al.* 2008; 2012), and that treatments applied after the infection window has closed provide little control, if any (Tegg *et al.* 2008). However, the present work suggests that treatments aimed to be applied at the beginning of the infection window may inadvertently miss the start of this period, that these treatments may be too late to provide control for all tubers, and that treatment made well prior to tubers entering the period of susceptibility, including applications immediately after emergence, gives a better result. This is presumably due to additional protection of those tubers that initiate earlier than anticipated.

Potato plants produce tubers asynchronously (Vreugdenhil & Struik 1989), which means that the time of both tuber initiation and the disease infection window varies for each developing tuber. Moreover, first tuber initiation is influenced by emergence date rather than planting date (Strunik *et al.* 1999), as well as cultivar genetics (Celis-Gamboa *et al.* 2003) and environmental factors such as day length. As such, the infection window of a crop will extend for a significant period of time, and may vary with date of emergence and other factors. It can also be difficult to determine precisely when first tuber initiation occurs, as tuber growth is not easily monitored and the time at which tuber initiation occurs doesn't relate to changes in plant physiology above ground (such as the number of leaves or flower development) (Celis-Gamboa *et al.* 2003). Field trials by McIntosh *et al*. of 3,5-D (1982) resulted in much lower disease control than previous glasshouse trials (McIntosh *et al.* 1981), which was suggested by the authors to be the result of extended tuber initiation in field conditions compared to in glasshouse grown plants, and a lack of persistence within the tuber of the 3,5-D. However, the successful disease control in one pot trial and two field trials in this study suggest that early applications are able to both cover

this extended period of tuber initiation in the field, and that reduced disease cover in field situations is likely a result of early initiating tubers not being protected. The timing of treatments to coincide with, or shortly after, first tuber initiation may be a risky practice given treatments applied after the onset of the infection window are ineffective in controlling common scab.

While the mechanism by which 2,4-D increases resistance in potato to common scab is as yet unknown, a relationship between auxin and thaxtomin has been demonstrated (Tegg *et al.* 2005; 2008; 2012). Tegg *et al*. (2005) found that an auxin sensitive mutant of *Arabidopsis thaliana* was more sensitive to thaxtomin A than the wildtype, while Tegg *et al*. (2008) observed that chlorosis and death induced by thaxtomin A was suppressed with the addition of the auxins 2,4-D and IAA. Tuber sensitivity to thaxtomin A has been shown in this and previous studies (Tegg *et al.* 2008; 2012) to be decreased with treatments of 2,4-D. Tegg *et al*. (2012) demonstrated a strong relationship between the suppression of thaxtomin A toxicity and disease control.

That 2,4-D treatments are more effective when applied prior to infection is consistent with examples of chemically induced SAR responses. For examples, SAR against fire blight in pears and apples induced by prohexadione-calcium, and SAR against late blight of potato induced by fosetyl-Al are both more effective when the treatments are applied earlier in the growing season. In both cases treatments are ineffective in inducing the SAR response if they are applied either late in the season, for control of late blight, or, in the case of fire blight, after symptom development has begun (Cooke & Little 2001; Schupp *et al.* 2002; Andreu *et al.* 2006). Other research has also suggested that the induction of SAR in potatoes against tuber diseases occurs more effectively when treatments are applied earlier in the growth stages (Bokshi *et al.* 2003; Cooke & Little 2001). While the suppression of common scab through 2,4-D treatments has been clearly associated with reduced severity of thaxtomin, the additional induction of a SAR response by 2,4-D treatments may also be possible.

The earliest spray treatments were included in this study to determine if sprays applied prior to tuber initiation would be effective in controlling disease by ensuring that the entire infection window was completely covered. It was expected that

treating plants even with low rates of a herbicide very early on in their development would be detrimental to the plant, and it wasn't known whether the 2,4-D would still be translocated to the tubers if it had been applied before they had developed. However, the results show that the 2,4-D applied soon after plant emergence (between 5-10 DAE) is retained within the plant until tuber initiation, translocated to the tubers once they have initiated and gives good disease control in comparison to sprays applied later (after tuber initiation has already occurred). Quantification of 2,4-D within tubers at harvest shows that plants treated with 2,4-D prior to tuber initiation had small amounts of 2,4-D within the tubers at harvest. This confirms that the 2,4-D is translocated to the tubers after they have initiated, and that sufficient 2,4-D stays within the tuber to provide control against disease through the infection window.

As well as ensuring active material is present in the plant prior to tuber initiation and in doing so providing improved disease control, treatments prior to tuber initiation allow for a longer period in which the 2,4-D can be metabolised within the plant, resulting in less 2,4-D within tubers at harvest when it is not needed and unwanted. The current maximum residue limit (MRL) for 2,4-D in potatoes is 100mg/g of fresh weight (FW) tubers. Quantification of 2,4-D in tubers from field trial #2 at harvest suggests that, when treated early with a low rate of 2,4-D, tubers would have a level of 2,4-D considered safe for human consumption, reaching levels of around a $1/10^{th}$ of the MRL. However, treatments of the same rate of 2,4-D applied later in the growing period, after tuber initiation, result in some tubers having 2,4-D levels near the MRL at harvest. This agrees with previous research that had shown that treatments of 2,4-D (at higher rates than those used in this study) targeted to the infection window resulted in levels of 2,4-D in tubers at harvest near or above the MRL (Tegg *et al.* 2008). The research presented in chapter 3 of this thesis demonstrated that foliar treatments of as little as 6.25mg/L 2,4-D are required for sufficient material to reach the developing tubers and control disease, and an increasing amount of 2,4-D within the tuber does not increase the toxin tolerance of the tuber past a certain level. Therefore, earlier treatments that result in these low levels of 2,4-D within the tubers during the period of susceptibility will protect the tubers through the infection window, and with natural metabolism of 2,4-D allow for tuber 2,4-D at harvest to reach levels well below the MRL. Increased levels of IAA

have been shown to decrease the amount of 2,4-D translocated to tubers (Burrell 1982), and as such the natural levels of IAA within the plant during different periods of growth may result in differing rates of translocation of 2,4-D from foliage to tubers, and therefore different levels of 2,4-D within tubers.

The success of these very early sprays led to the hypothesis that the 2,4-D could be applied even earlier, before planting, as a treatment applied directly to the seed tubers. This is examined in Chapter 4.

2.5.2 Single sprays as effective as multiple sprays in controlling disease

The current study suggests that single applications are as equally effective in controlling disease as double or triple spray treatments. This is in contrast with previous work by Tegg *et al*. (2008), which found that multiple sprays resulted in lower disease severity than single sprays. Chemically induced resistances can require multiple applications throughout the growing season to achieve disease control. The resistance of potato to late blight induced by foliar applied phosphorous acid was found to be three times more effective from double and triple applications applied at 14 day intervals than single applications (Taylor *et al.* 2011). However, the induced control can be achieved with a single targeted application, such as the control achieved by a single application of benzothiadiazole, which induces resistance against post-harvest melon diseases and achieves similar levels of disease control as four applications made throughout the growing season (Bokshi *et al.* 2006). That single applications early in the growing season resulted in excellent disease control and low levels of 2,4-D in the tuber at harvest further suggests that one targeted application provided enough material to the developing tubers to effectively control common scab, and that multiple sprays spread across the growing season are not required to "top-up" active materials. Additional sprays would likely be applied after tubers are susceptible, and this research has suggested they would be less effective.

2.5.3 Agronomic effects

The 2,4-D treatments in this study were not shown to have a significant effect on the mean number of tubers or mean mass of tubers in any trials. There was an effect found from treatments on total tuber mass in one field trial and both pot trials, however the effect was not consistent between trials, with treatments resulting in

both higher and lower total tuber masses when compared with controls across the trials. In both pot trials there was a trend towards earlier sprays having a lower total tuber mass, and treatments applied around tuber initiation in pot trial #2 resulted in the lowest tuber masses. This is a similar result to that of McIntosh *et al.* (1981), who observed that treatments applied at 21 DAE resulted in increased phytotoxic effects. Treatments prior to tuber initiation were not included in the McIntosh *et al.* (1981) trials. Tegg *et al*. (2008) also observed reduced total tuber weight in pot trials from treatments applied at a similar date. It may be that 2,4-D applications targeted towards the beginning of the infection window, when tubers are initiating, has a greater effect on the total tuber mass of a plant than treatments applied prior to tuber initiation, or after tubers have finished rapidly growing, possibly as a result of changes in the hormone balance (Strunik *et al.* 1999).

In interpretation of these results we need to acknowledge the main aim of this study was to determine the efficacy of 2,4-D as a disease control tool, and these trials were conducted to maximise disease levels with plants grown under continuous water stress. As a result some agronomic outcomes, such as tuber size, tuber number, and yield are not indicative of what would occur in a commercial crop, grown to maximise yield and productivity. Pot trials can also be poor indicators of agronomic performance in potato crops, due to restrictions on root and tuber development, and care must be taken in the interpretation of these data (Wilson *et al.* 2010). To determine more accurately the agronomic effects of these treatments, further trials would need to be undertaken using commercial growing practices, with standard irrigation and other management practices in place.

2.5.4 Tuber slice assays

Tegg *et al*. (2012) found a consistent relationship between the suppression of common scab disease and the thaxtomin A sensitivity of the tuber in plants that had been treated with a range of chemicals, including 2,4-D, demonstrating that the suppression of thaxtomin toxicity resulted in disease control. The authors concluded that the tuber slice assay was a good determinant of the level of disease control that is induced by chemicals that affect thaxtomin A toxicity. However, as 2,4-D is naturally metabolised within the tuber (Burrell 1982), which decreased tuber 2,4-D levels over time, the toxin-tolerance of the tuber is lower at harvest than both after

treatment with 2,4-D and when the tuber is susceptible to disease. Tuber slice assays of harvested tubers therefore give a less accurate indication of how toxin-tolerant the tuber was during the period of susceptibility to infection. For example, a high application rate of 2,4-D resulting in a large amount of 2,4-D in the tuber at harvest will give a lower necrosis rating in the tuber slice assay, while a late treatment, which leaves less time for the 2,4-D in the tuber to be metabolised, may result in a similarly high level of 2,4-D in the tuber at harvest, and give a similarly low necrosis scores in the tuber slice assay. However, these two application strategies will result in very different disease control efficacies. Inversely, a smaller amount of 2,4-D applied early in the season will result in lower levels of 2,4-D within the tuber at harvest, and give higher necrosis scores in the tuber slice assay, yet this treatment may provide a similar level of disease control as a higher application rate, and a greater level of disease control than a later treatment. Both previous research (Tegg *et al.* 2008) and the results presented in this thesis suggest that higher levels of 2,4-D within the tuber do not necessarily give greater disease control, and late sprays can often give no disease control if applied after the infection window. The tuber slice assay, therefore, needs to be interpreted with rate and timing of the treatment, and date of harvest in mind.

The tuber slice assay for assessing toxin tolerance is a good indication of the levels of 2,4-D in the tubers at harvest, and is a valid and useful test (Tegg *et al.* 2012), but tuber slice assays at the time of harvest are not necessarily a good determinate of the levels of 2,4-D in the tuber at the time of infection or an indication of whether the tuber is protected during the infection window. Ideally the tuber slice assay should either be undertaken on tubers harvested while they are still susceptible to infection, or in conjunction with disease assessments and 2,4-D quantification. However, accurate quantification of 2,4-D within tubers can be both time-consuming and expensive. The tuber slice assay method may be used as a more efficient and cost effective method of screening tubers when a comparative indication of disease resistance is required.

2.5.5 Controlling common scab may result in reduced powdery scab

The correlation between the tuber surface coverage by lesions of common scab and powdery scab, caused by *Spongospora subterranea* f. sp. *subterranea* found in field trial #1 (Table 3.4) is an interesting result that suggests there may be some interaction between the two diseases. There was a low incidence of powdery scab in the following season's field trial, which was not assessable, and as such there is no direct replication for this data. However, there was a correlation found between common scab and powdery scab lesion surface coverage in the field trial detailed in chapter 3, which focused on application rates rather than timing of application (Figure 3.1 on page 89). While 2,4-D appears to reduce common scab through a thaxtomin specific response, which would be unlikely to have an effect on the powdery scab pathogen, it is also possible that 2,4-D treatments may also induce a SAR response, such as the production of reactive oxygen species, which may also provide some resistance to powdery scab. Treatments of IAA in potato have been shown to result in an increased transcription of auxin related stress related proteins, which also increase in response to fungal infection, and may have a role in plant defence responses (Zanetti *et al.* 2003).

By controlling common scab, and therefore preventing the development of lesions, the size and number of entry sites for the powdery scab pathogen to enter may be reduced. This could result in a reduction of both the number of powdery scab lesions and the amount of powdery scab colonisation on the tuber. There has been little research to date on the interaction between common and powdery scab, despite importance to potato production, as they tend to be studied in isolation. Indeed one common scab control method, targeted irrigation, may encourage the powdery scab pathogen through the creation of a more suitable environment, and its use for controlling common scab may increase powdery scab. Delayed planting, another control method of common scab, has also been found to reduce powdery scab incidence, if the later planting date allows soils to have warmed beyond temperatures acceptable to the pathogen (Falloon 2008).

Controlling common scab through the application of 2,4-D has not been shown to create a change in the pathogen environment outside of the tuber, and it is possible that it is a control method for common scab that either does not increase the risk of,

or possibly reduces the risk of powdery scab. If further data on the interaction between the two pathogens supported this hypothesis, 2,4-D (or similar chemicals) may indeed be a possible control method for both diseases, if common scab is present. Waterer (2010) found the opposite effect on tubers sprayed with 2,4-D, observing that 2,4-D treatments tended to decrease common scab but slightly increase powdery scab disease levels, and suggested that this may be a result of reduced competition for entry sites.

It is worth noting that this observed correlation may also be a result of the visual estimation of common scab symptoms being conflated by the presence of powdery scab lesions. Care was taken by assessors to identify features of the different diseases, such as sporeballs inside powdery scab lesions (Harrison *et al.* 1997). However, due to the inaccurate nature of visual symptom scoring, and the similarity between the lesions of the two diseases, higher levels of one disease may have led to higher levels of the other disease being estimated. The use of molecular diagnostic techniques would be able to provide more accurate results (Qu *et al.* 2011).

2.6 Conclusion

This research demonstrated the importance of timing of applications in the effectiveness of 2,4-D induced resistance to common scab of potato. It has shown that foliar treatments of 2,4-D applied as early as five days after crop emergence can provide effective control in potatoes against common scab disease, and suggests that applications prior to tuber initiation are more effective in controlling disease than applications timed to coincide with, or after tuber initiation. These early treatments appear to provide sufficient 2,4-D to the tuber to control disease through the infection window, while also allowing sufficient time for the amount of 2,4-D within the tuber to reach acceptable levels well below the MRL by harvest. Additionally, this research has demonstrated that single, early applications are as effective in controlling disease as multiple applications.

Chapter 3: Determination of optimal rate of foliar 2,4-D application for common scab control in potato

3.1 Abstract

Previous research had found that to provide effective disease control, foliar applications of 2,4-D was required at concentrations near those used for herbicidal means, and thus resulted in phytotoxic side effects on an increase in tuber deformity and yield reductions. This study examines the effect of a range of application rates on disease control, in Russet Burbank and Desiree cultivars, and presents the result of two pot trials and one field trial. The data obtained suggests that much lower rates than had been previously used, as low as 1.625mg/L, provided some degree of disease control and suppressed the toxicity of the phytotoxin thaxtomin, and that an application of 25mg/L provided disease control as consistently and effectively as the previously studied rates, without the associated phytotoxic effects. These lower application rates also resulted in levels of 2,4-D within tubers at harvest far below the MRL.

3.2 Introduction

Common scab, caused by pathogenic *Streptomyces* spp. is an economically important disease of potato found in all potato growing regions (Loria *et al.* 2006). Essential to (Kers *et al.* 2005), and correlated with (Loria *et al.* 1995; Goyer *et al.* 1998; King *et al.* 1991) the pathogenicity of *Streptomyces* spp. is the production of the phytotoxins, thaxtomins (King *et al.* 1989). Thaxtomins have been demonstrated to reproduce common scab disease symptoms on potato tubers in the absence of the pathogen (Lawrence *et al.* 1990; Leiner *et al.* 1996). Management of common scab is partly achieved through a combination of control methods, but there are no reliable, effective management strategies available. Management strategies include delayed planting (Waterer 2002a), the use of moderately resistant varieties (Wilson *et al.* 2009; Hiltunen *et al.* 2011), and targeted irrigation during tuber development (Lapwood *et al.* 1973). Seed tuber treatments and soil amendments have been shown to be effective control methods (Wilson *et al.* 1999), as have some biological controls (Schottel *et al.* 2001; Neeno-Eckwall *et al.* 2001; Han *et al.* 2005; Jobin *et al.* 2005).

McIntosh et al. (1981; 1982), through testing a wide range of chemicals for their effect on common scab suppression, found that 2,4-D and 3,5-D were both effective in reducing common scab of potato. Applications of 200mg/L of 2,4-D and 3,5-D reduced the development of common scab symptoms by 50% and 90% respectively, compared to control treatments (McIntosh *et al.* 1981). At the rates applied 2,4-D caused an increased number of deformed tubers, increased overall tuber numbers, and decreased tuber size. The phytotoxic effects of 3,5-D were less severe than those of 2,4-D, but 3,5-D was found to be less effective in field trials, with reductions of only 30% (McIntosh *et al.* 1982). Most commonly used as a herbicide, 2,4-D is a phytotoxic, synthetic auxin. It is systemic, with 2,4-D applied to foliage being translocated through the plant. When applied to the leaves of potato plants, it is translocated to the tubers where it is metabolised (Burrell 1982). Recommended herbicidal application rates range from a minimum concentration of approximately 680mg/L applied until runoff for lawns (Nufarm Australia Limited 2011).

Early Australian research (Tegg 2006) into 2,4-D induced resistance of common scab of potato used a rate of application the same as that used by McIntosh et al. (1981),

of 200mg/L 2,4-D, applied up to three times to the one crop. It was found to be effective but still resulted in an increase in tuber deformation and yield reduction. Further research (Tegg *et al.* 2008) found that a lower application rate of 50mg/L 2,4-D, a quarter of the rate used previously, still provided similar levels of induced toxin tolerance to those observed from the 200mg/L rate. Additionally, this rate was still effective from a single application, a reduction of up to a sixth of the original amount of 2,4-D applied in previous trials. However, at these rates, levels of 2,4-D in the tuber six weeks after application were still above the Australian maximum residue level (MRL), of 100ng/g of tuber fresh weight (Commonwealth of Australia 2011), with residue levels increasing with application rate.

This chapter aims to determine the relationship between the rate of 2,4-D application and resultant disease control; to determine the level at which 2,4-D is no longer effective at controlling disease; to determine the effect of rate on the phytotoxicity of 2,4-D; and to determine the optimal rate that achieves the maximum possible disease control with the lowest, yet still practical, spray concentration.

3.3 Materials and Methods

3.3.1 Inoculum preparation for pot trials

Pathogenic *Streptomyces scabies* strain G#20, initially isolated from a common scab infected tuber harvested from north-west Tasmanian in 1990, was used in these experiments. The isolate was grown on 10mL ISP2 (Shirling & Gottlieb 1966) agar slopes (10g/L malt extract, 4g/L yeast extract, 4g/L glucose, 12g/L agar, pH 7.3) until sporulation. Colonised agar slopes were then aseptically transferred to a sterilised mixture of 120g vermiculite and 500mL SAY solution (20g/L sucrose, 1.2g/L *L*-asparagine, 0.6g/L K_2HPO_4, 10g/L yeast extract, pH 7.2) (Labruyère 1971). The inoculum was incubated in the dark at 24ºC until profuse sporulation was observed at around 14 days. Approximately 1L of colonised vermiculite inoculum was added to 25L of potting mix containing sand, peat and composted pine bark at a ratio of 10:10:80, at pH 6.0, premixed with Osmocote 16:3.5:10 N:P:K resin coated fertiliser (Scotts Australia Pty Ltd.) at the rate of 6kg/m^3, and thoroughly mixed using a cement mixer. The inoculum containing potting mix was then used to fill plastic planter bags of 5L capacity (200 x 200mm, Botany Horticultural, Queensland, Australia).

3.3.2 Planting material

A fortnight prior to planting, potato tubers of the varieties Russet Burbank and Desiree were removed from cold storage, where they had been stored since harvesting approximately 6 months before. For the pot trials tubers were disease free mini-tubers from glasshouse grown tissue culture plantlets. In trial #1 the tubers were cut into approximately 15g pieces and left to suberise before planting. In trial #2 15g tubers were used and were planted whole. For the field trial tubers were visibly clean certified seed tubers.

3.3.3 Preparation of 2,4-D treatments

3.3.3.1 Pot trials

2,4-D solutions were prepared by dissolving 400mg of crystalline 2,4-D (Sigma Aldrich, St. Louis, USA) in approximately 10mL of 70% ethanol over heat. This solution was then mixed with 1L of warm water to produce a 400mg/L solution. 0.5L of this 400mg/L solution was retained, and the other 0.5L was added to 0.5L of warm water to produce a 200mg/L solution. This dilution series was continued until a 6.25mg/L solution was produced. A control treatment of warm water only was used. Warm water was used for all dilutions to assist in keeping the 2,4-D in solution. Tween-80 was added at a rate of 0.5g/L to all 2,4-D and control treatments as a wetting agent to assist foliar application.

3.3.3.2 Field trial

As the spray treatments would be applied from a backpack spraying rig in close proximity to other crops, and in accordance with licenses, the less volatile amine form of 2,4-D was used to minimise the chance of damage to nearby horticultural crops. The commercial herbicide Amicide 625 (Nufarm Ltd., Victoria, Australia) with 625g/L 2,4-D present as dimethylamine and diethanolamine salts was used, and it was ensured that an equivalent amount of the active 2,4-D molecule was present compared with that used in the pot trials. To prepare the spray treatments, 4.4mL of Amicide 625 was dissolved in 1L of water, with 0.5g of Tween-80 as a wetting agent. This 4.4mL/L Amicide solution was then mixed with 4L of water within the backpack spraying rig to make a 880µL/L solution. This was determined to be the equivalent of a 400mg/L 2,4-D solution. All spray concentrations were prepared in this manner, according to the following dilution table:

Amicide 625	**Water**	**Conc.**	**2,4-D Conc.**
4400µL	5L	880µL/L	400mg/L
1100µL	5L	220µL/L	100mg/L
275µL	5L	55µL/L	25mg/L
69µL	5L	13.8µL/L	6.25mg/L
17µL	5L	3.4µL/L	1.625mg/L

The spray rig was washed with water between the applications of different treatments. Treatments were applied from the lowest rate to highest. The control treatment was applied with a clean handheld sprayer. The spray was applied to the foliage until run off occurred.

3.3.4 Pot trials

Two pot trials testing varied rates of 2,4-D foliar treatments were conducted.

3.3.4.1 Pot trial #1 (2008/09)

The first pot trial was planted on 11th December, 2008. The two potato varieties used possessed moderate resistance (Russet Burbank) and moderate susceptibility (Desiree) to common scab. Plants emerged on 21st December, 2008. There were eight spray treatments of varying concentrations of 2,4-D: 400mg/L, 200mg/L, 100mg/L, 50mg/L, 25mg/L, 12.5mg/L, 6.25mg/L 2,4-D and a water control treatment with no 2,4-D. The treatments were applied as single sprays. Each treatment was applied to five pots of each variety (80 pots in total), with pots arranged in a completely randomised design.

The spray treatments were timed to coincide with the critical infection period, which occurs 1 to 2.5 weeks after tuber initiation (Adams & Lapwood 1978). The spray treatments were applied 38 days after emergence (DAE) on 27th January, 2009. Prior to application of each treatment, plants were removed to a separate area to prevent spray drift onto plants of different treatments. The sprays were applied to the foliage as a fine mist until run-off occurred. The spray was allowed to dry before the plants were returned to the glasshouse. All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease (Lapwood & Hering 1970). No other pesticides were applied. Plants were grown under glasshouse conditions, with the temperature maintained at 25-30ºC. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to

each pot. Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 1st April, 2009. Tubers were stored at 4ºC in plastic netted bags.

Soil was brushed from tubers, which were then assessed for common scab disease severity using the methods described in section 2.3.6. Tuber number and mass (fresh weight) were measured. Any tuber disfigurements were noted. Sensitivity to thaxtomin A was assessed in two tubers from each pot. Tubers without obvious lesions were chosen and assayed following the method described in section 2.3.7.

3.3.4.2 Pot trial #2 (2009/10)

The second pot trial was planted on 23rd December 2009. Treatments repeated those used in the trial #1. Plants emerged on 2nd January 2010. Plants were maintained as per trial #1, but were grown outside on a concrete slab and subject to natural weather events and temperatures. Irrigation was applied as required ensuring soil dried between watering events. Pots had additional inoculated vermiculate and potting mix added on 22DAE. Tubers were harvested, following plant senescence, on 13th May 2010. Disease, yield and toxin sensitivity assessments were as per trial #1. The levels of 2,4-D within selected tubers at harvest were quantified using the method described in section 2.3.8.

3.3.5 Field trial #1

One field trial further testing the effect of varying rates of 2,4-D foliar treatments was conducted. The trial site was located upon *Forester Lodge*, the property of Mr. Wilson Geale at Waterhouse in North East Tasmania. The soil was predominately sandy. The immediate area had been sown with a trial potato crop in the previous season (Field Trial #2 in section 2.3.5.2), and a commercial processing potato crop in the season prior to that. The surrounding area had been sown with an oil seed crop in the previous season and a lucerne feed crop in the trial season.

The trial was planted on 27th October, 2010. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants of the same variety, and plots consisting of two subplots, one each variety. To ease planting the subplots were not randomised within each plot. The trial was replicated four times. A visually distinct variety was planted as a buffer at the edge of the trial, between each treatment row, and between treatment plots within each row. The average emergence date was estimated to be 20th November 2010. Plants were treated on 9th December 2010 at 20 DAE. Six different spray concentration treatments were applied: 1.625mg/L, 6.25mg/L, 25mg/L, 100mg/L, and 400mg/L 2,4-D equivalence, and a control spray containing no 2,4-D. All treatments were single sprays, and all were applied on the same date.

The trial was watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required. After senescence the trial was sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically on 4th April, 2011. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4ºC prior to assessment. Before assessment the tubers were washed to remove soil and then weighed to determine yield for each plot (the combined tubers from five plants).

Each tuber was then assessed for common scab severity (DCS and LDS), and powdery scab severity (DCS), using the scoring methods described in section 2.3.6. Tuber thaxtomin sensitivity was assessed using the method described in section 2.3.7 modified so that tubers were taken from replicates 1 and 2 of the trial, and two tubers

were assayed from each replicate (one tuber per Petri dish), with eight filter paper discs distributed over the slices from each tuber. A total of 32 filter paper discs were spread over four tubers from two replicates in four Petri dishes, for each treatment.

3.3.6 Data analysis

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009). Multivariate Analysis of Variance was used to determine significant effects from, and interactions between treatment factors. Probabilities less than 0.05 were considered to be significant and Fischer's least significant difference (LSD) test was used for comparison of treatment means. Linear Regressions were used to examine the relationship between powdery scab and common scab severity.

3.4 Results

3.4.1 Disease control

3.4.1.1 Pot trials

In pot trial #1 there was no significant effect found from spray rate on either measure of disease severity (Table 3.1). However, though not significant, all treatments did result in lower disease coverage than the control treatment, except the Desiree treatment of 200mg/L. In Russet Burbank, all treatments of 25mg/L 2,4-D and above had no diseased tubers. There was a significant effect found from spray rate on disease incidence, measured as the proportion of diseased tubers per pot, with all treatments resulting in a significantly lower disease incidence than the control.

In pot trial #2 all 2,4-D treatments had significantly lower DCS than the control treatment (Table 3.1). The lowest (6.25mg/L 2,4-D) and highest (400mg/L 2,4-D) treatments had significantly lower DCS than the 12.5mg/L treatment, and there were no other significant differences between treatments. All treatments except the 12.5mg/L and 50mg/L treatments had significantly lower lesion depth scores than the control treatment. The 6.25mg/L and 400mg/L treatments had significantly lower LDS than these treatments, and the 6.25mg/L treatment also had significantly lower LDS than the 25mg/L treatment. There were no other significant differences between treatments. There was a significant effect found from spray rate on the number of lesions per tuber. The 6.25mg/L, 25mg/L, 100mg/L and 400mg/L treatments had significantly fewer lesions per tuber (of tubers with any lesions) than the control treatment, and the 6.25mg/L and 400mg/L treatments also had significantly fewer lesions per tuber than the 12.5mg/l treatment. All other treatments were not significantly different (Table 3.1)

There was a significant effect found from spray rate on the proportion of diseased tubers. All treatments resulted in significantly less diseased tubers than the control. The 6.25mg/L and 400mg/L treatments had the lowest proportion of diseased tubers, and were significantly lower than the 12.5mg/L treatment. All other treatments were not significantly different. For all trials and all measures of disease severity and incidence there was a significant effect found from variety. Russet Burbank had lower disease cover and proportion of disease tubers than Desiree in both trials.

Russet Burbank had significantly deeper lesions than Desiree in pot trial #1, but significantly shallower lesions than Desiree in pot trial #2. There were no interactions between spray rate and variety. (Table 3.1)

Table 3.1 Pot trials The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants, on common scab disease severity measured a) as the area of tuber surface covered by scab lesions measured using the Disease Cover Score (section 2.3.6), b) derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using the Lesion Depth Score (section 2.3.6) and on incidence measured as incidence measured as the number of tubers with visible lesions as a proportion of total tubers per pot. **Pot trial #1** There was a significant effect found on proportion of diseased tubers from spray rate ($p < 0.001$, SED = 9.1, LSD = 18.21). There was no significant effect found from spray rate on DCS or LDS. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.172, LSD = 0.344), LDS ($p < 0.001$, SED = 0.211, LSD = 0.421) and proportion of diseased tubers from spray rate ($p < 0.001$, SED = 9.1, LSD = 18.21) and variety ($p < 0.001$, SED = 4.55, LSD = 9.11). **Pot trial #2** There was a significant effect found from spray rate on DCS ($p = 0.001$, SED = 0.215, LSD = 0.43), LDS ($p = 0.002$, SED = 0.25, LSD = 0.499) and proportion of diseased tubers ($p = 0.001$, SED= 5.66, LSD = 11.31). There was a significant effect found from variety on DCS ($p = 0.001$, SED = 0.108, LSD = 0.215), LDS ($p < 0.001$, SED = 0.125, LSD = 0.25), and proportion of diseased tubers ($p = 0.042$, SED = 2.83, LSD = 5.66). There were no significant interactions between factors. Treatments with the same letter, in the same column and trial, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Trial	Spray Rate (mg/L 2,4-D)	Disease Cover Score (DCS) (and Cover (%))			Lesion Depth Score (LDS)			Prop. of Diseased Tubers/Pot (%)		
		R.Burbank	Desiree	Mean (Rate)	R.Burbank	Desiree	Mean (Rate)	R.Burbank	Desiree	Mean (Rate)
Pot Trial #1	**0**	0.6 (1.8%)	1.4 (6.9%)	1.1	0.6	2.0	1.3	40.0	58.7	49.3 c
	6.25	0.8 (4.6%)	0.8 (2.7%)	0.8	0.8	0.9	0.9	9.0	16.7	14.3 ab
	12.5	0.4 (1.5%)	0.4 (1.5%)	0.4	0.4	0.8	0.6	6.7	10.0	8.3 ab
	25	0.0	1.0 (5.5%)	0.5	0.0	1.0	0.5	0.0	8.3	4.2 ab
	50	0.0	1.3 (5.2%)	0.7	0.0	1.4	0.7	0.0	43.3	21.7 b
	100	0.0	0.8 (4.3%)	0.4	0.0	1.1	0.5	0.0	32.0	16.0 ab
	200	0.0	1.8 (6.6%)	0.9	0.0	2.4	1.2	0.0	29.7	14.8 ab
	400	0.0	0.4 (1.5%)	0.2	0.0	0.4	0.2	0.0	5.0	2.5 a
	Mean (Variety)	0.2 a	1.0 b		1.0 a	0.2 b		7.0 a	25.8 b	
Pot Trial #2	**0**	0.9 (2.6%)	1.2 (3.9%)	1.0 c	0.9	1.4	1.2 d	29.7	24.5	27.1 c
	6.25	0.1 (0.1%)	0.0	0.1 a	0.2	0.0	0.1 a	4.0	0.0	2.0 a
	12.5	0.7 (2.2%)	0.7 (2.2%)	0.7 bc	0.6	0.8	0.7 cd	15.2	13.3	14.3 b
	25	0.1 (0.1%)	0.8 (2.7%)	0.5 ab	0.2	1.0	0.6 bc	5.0	7.6	6.3 ab
	50	0.1 (0.1%)	0.7 (1.5%)	0.4 ab	0.2	1.4	0.8 cd	3.3	17.2	10.3 ab
	100	0.2 (0.6%)	0.4 (0.8%)	0.3 ab	0.2	0.6	0.5 abc	2.5	15.4	8.9 ab
	200	0.0	0.8 (4.1%)	0.4 ab	0.0	0.9	0.4 abc	0.0	23.2	11.6 ab
	400	0.0	0.4 (1.2%)	0.2 a	0.0	0.4	0.2 ab	0.0	5.5	2.8 a
	Mean (Variety)	0.3 a	0.6 b		0.3 a	0.8 b		7.5 a	13.3 b	

3.4.1.2 Field trial

There was no overall significant effect found from spray rate on disease severity measured either as disease cover or lesion depth in the field trial (Table 3.2). However, there is a trend in Russet Burbank towards a reduction in DCS with increasing spray rate treatments, with the 1.625mg/L treatment having slightly less disease cover than the control, and the other 2,4-D spray treatments having a similarly greater reduction.

Table 3.2 Field trial The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants, on common scab disease severity measured a) as the area of tuber surface covered by scab lesions measured using the Disease Cover Score (section 2.3.6), b) derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using the Lesion Depth Score (section 2.3.6). There was no significant found from spray rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.0805, LSD = 0.1610) and LDS ($p < 0.001$, SED = 0.211, LSD = 0.421). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Spray Rate (mg/L 2,4-D)	Disease Cover Score (DCS) and Percentage Cover (%)			Lesion Depth Score (LDS)		
	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
0	1.21 (4.29%)	1.55 (6.15%)	1.32	1.11	1.58	1.27
1.625	0.90 (2.44%)	1.94 (10.68%)	1.42	1.17	2.21	1.69
6.25	0.63 (1.16%)	1.74 (9.13%)	1.19	1.00	2.07	1.53
25	0.72 (1.57%)	2.09 (10.53%)	1.40	1.03	2.24	1.63
100	0.63 (1.17%)	0.94 (2.65%)	0.76	1.02	1.31	1.15
400	0.60 (1.06%)	2.15 (12.21%)	1.26	1.07	2.22	1.56
Mean (Variety)	0.78 a	1.75 b		1.07 a	1.98 b	

There was no significant effect found from spray rate on powdery scab disease severity (Table 3.3). Russet Burbank had significantly lower severity of both common scab and powdery scab disease than Desiree. However, there was a strong correlation ($r^2 = 0.746$) between common scab and powdery scab cover (Figure 3.1).

Table 3.3 Field trial The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants, on powdery scab disease severity measured a) as the area of tuber surface covered by scab lesions measured using the Disease Cover Score (section 2.3.6) b) derived percentage cover (ANOVA not performed on this data). There was no significant found from spray rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.121, LSD = 0.248). There was no significant interaction between factors. Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Spray Rate (mg/L 2,4-D)	**Powdery Scab Disease Disease Cover Score (DCS) and Percentage Cover (%)**		
	Russet Burbank	**Desiree**	**Mean (Rate)**
0	1.54 (6.57%)	1.77 (7.13%)	1.62
1.625	1.26 (4.56%)	2.21 (11.34%)	1.74
6.25	1.12 (4.04%)	1.90 (10.92%)	1.51
25	1.33 (5.33%)	2.75 (17.95%)	2.04
100	0.90 (2.50%)	1.77 (8.16%)	1.28
400	1.10 (3.84%)	2.26 (11.91%)	1.60
Mean (Variety)	1.21 a	2.12 b	

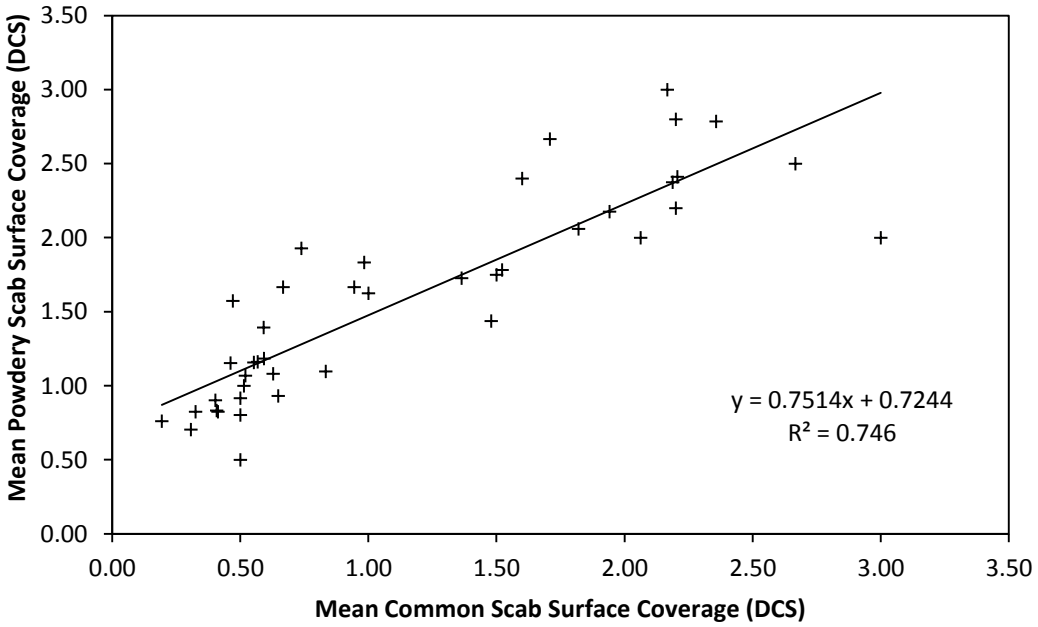

Figure 3.1 Field trial Linear regression between the mean surface coverage of common scab and powdery scab, on tubers harvested from Russet Burbank and Desiree plants treated with 2,4-D at various rates. $R^2 = 0.746$.

3.4.3 Agronomic effects

3.4.3.1 Pot trials

There was no significant effect found from spray rate on total tuber mass, mean mass per tuber, or number of tubers in either pot trial. However, in pot trial #1 the control treatment did have the greatest tuber mass of all the treatments (Table 3.4), and in pot trial #2 the control treatment had one of the greatest tuber masses of all the treatments, and the greatest mass per tuber of all the treatments (Table 3.5). There was no trend apparent for number of tubers in either trial. Russet Burbank had a significantly lower mean total tuber mass and mean number of tubers than Desiree in both trials, and a significantly higher mean mass per tuber in pot trial #2. Variety was not found to have a significant effect on mean mass per tuber in pot trial #1.

3.4.3.2 Field trial

Spray rate was not found to have a significant effect on total tuber mass, mean mass per tuber, or number of tubers in the field trial, and there was no apparent trend in any of these measures of agronomic performance. Russet Burbank had a significantly higher total tuber mass and number of tubers than Desiree, and a significantly lower mean mass per tuber (Table 3.5).

Table 3.4 Pot trials The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants on mean total tuber mass, mean mass per tuber, and mean number of tubers. **Pot trial #1** There was a significant effect found from variety on mean total mass ($p < 0.001$, SED = 2.83, LSD = 5.67), and mean number of tubers ($p = 0.011$, SED = 0.342, LSD = 0.683) **Pot trial #2** There was a significant effect found from variety on mean total mass ($p = 0.002$, SED = 2.87, LSD = 5.75), mean mass per tuber ($p = 0.004$, SED = 1.083, LSD = 2.166) and mean number of tubers ($p = 0.004$, SED = 0.536, LSD = 1.072). There was no significant effect found from spray rate. There were no significant interactions between factors. Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Trial	Spray Rate (mg/L 2,4-D)	Mean Total Tuber Mass (g)			Mean Mass/Tuber (g)			Mean Number of Tubers		
		Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
Pot Trial #1	**0**	27.8	54.9	41.3	17.1	23.8	20.5	2.6	3.6	3.1
	6.25	24.0	31.2	27.6	10.6	9.2	9.9	3.4	3.6	3.5
	12.5	23.0	56.8	39.9	9.6	21.9	15.7	2.8	3.4	3.1
	25	21.1	47.6	34.3	7.3	10.1	8.7	3.2	4.8	4
	50	25.1	48.0	36.6	11.5	14.0	12.8	2.4	3.4	2.9
	100	27.6	44.3	36.0	21.4	25.1	23.3	1.8	2.4	2.1
	200	29.7	44.2	36.9	12.1	13.9	13.0	2.6	3.8	3.2
	400	25.1	30.8	27.9	14.5	13.3	13.9	2.6	3.6	3.1
	Mean (Variety)	25.4 a	44.7 b		13.0	16.4		2.7 a	3.6 b	
Pot Trial #2	**0**	57.0	75.8	66.4	17.7	9.3	13.5	4.6	8.6	6.6
	6.25	59.9	68.3	64.1	13.1	10.8	11.9	5.2	7.6	6.4
	12.5	64.3	69.1	66.7	14.6	8.9	11.7	5.2	8.2	6.7
	25	63.5	69.0	66.3	13.9	9.1	11.5	4.8	8.6	6.7
	50	51.9	69.9	60.9	10.1	11.5	10.8	5.2	6.4	5.8
	100	57.0	68.0	62.5	9.1	10.0	9.5	6.8	6.8	6.8
	200	59.6	60.7	60.1	11.8	7.6	9.7	5.4	8.4	6.9
	400	51.0	59.7	55.3	9.7	6.6	8.2	6	9.6	7.8
	Mean (Variety)	58.0 a	67.6 b		12.5 a	9.2 b		5.4 a	8.0 b	

Table 3.5 Field trial The effect of 2,4-D applied as single sprays at various rates to the foliage of Russet Burbank and Desiree plants on mean total tuber mass, mean mass per tuber, and mean number of tubers. There was a significant effect found from variety on mean total mass ($p < 0.001$, SED = 280.6, LSD = 575.8), mean mass per tuber ($p < 0.001$, SED = 8.57, LSD = 17.59) and mean number of tubers ($p < 0.011$, SED = 3.64, LSD = 7.41) There was no significant effect found from spray rate. There were no significant interactions between factors. Treatments with the same letter, in the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate	Mean Total Tuber Mass (g)			Mean Mass/Tuber (g)			Mean Number of Tubers		
(mg/L 2,4-D)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
0	3130	1275	2512	74.3	90.0	79.6	42.0	6.8	24.4
1.625	2022	798	1410	76.2	143.4	109.8	30.8	6.8	18.8
6.25	3301	939	2120	75.1	109.5	92.3	44.5	7.0	25.8
25	3742	1107	2424	80.7	97.7	89.2	33.3	7.8	20.5
100	2755	1310	2136	79.8	100.5	88.7	34.5	9.5	22.0
400	2961	1450	2314	69.5	132.6	96.5	44.8	8.3	26.5
Mean (Variety)	2952 a	1110 b		75.7 a	114.9 b		38.3 a	7.7 b	

Table 3.6 The effect of 2,4-D foliar sprays on mean necrosis levels from the treatment of tuber slices with thaxtomin A. 2,4-D foliar sprays were applied at various rates to the foliage of Russet Burbank and Desiree plants. Necrosis was rated using the scale described in section 2.3.7. There was an interaction found between spray rate and variety in Pot trial #1 ($p = 0.001$, SED = 0.1357, LSD = 0.2669), Pot trial #2 ($p < 0.001$, SED = 0.089, LSD = 0.1745) and Field trial ($p = 0.027$, SED = 0.1125, LSD = 0.2213). Treatments with the same letter, within each variety and trial, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate	Pot Trial #1		Pot Trial #2		Field Trial	
(mg/L 2,4-D)	Russet Burbank	Desiree	Russet Burbank	Desiree	Russet Burbank	Desiree
0	2.73 a	2.50 a	2.38 a	1.70 a	1.92 a	1.80 a
1.625					1.66 b	1.25 b
6.25	2.04 bc	2.35 a	1.88 b	1.54 ab	1.38 c	0.95 c
12.5	2.10 b	1.63 bc	1.66 c	1.42 bc		
25	1.96 bcd	1.48 bc	1.50 cd	1.28 cd	1.06 d	1.05 bc
50	1.90 bcd	1.69 b	1.55 cd	1.43 bc		
100	1.85 bcd	1.60 bc	1.60 cd	1.10 ef	1.33 c	1.27 b
200	1.71 d	1.42 c	1.42 d	1.03 f		
400	1.81 cd	1.73 b	1.17 e	1.21 de	1.41 c	1.08 bc

3.4.4 Toxin tolerance

3.4.4.1 Pot trial #1

There was a significant interaction between variety and spray rate on necrosis in pot trial #1 (Table 3.6). In Russet Burbank all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control. The 12.5mg/L treatment had significantly higher necrosis scores than the 200mg/L and 400mg/L treatments, and the 6.25mg/L treatment had significantly higher necrosis scores than the 200mg/L treatment. All other treatments were not significantly different from each other. In Desiree all treatments except the 6.25mg/L treatment resulted in significantly higher toxin tolerance than the control. The 6.25mg/L treatment had significantly higher necrosis scores than all the other treatments, except the control treatment, which it was not significantly different to. The 50mg/L treatment had significantly higher necrosis scores than the 200mg/L treatment. All other treatments were not significantly different from each other. (Table 3.6)

3.4.4.2 Pot trial #2

As in pot trial #1, there was a significant interaction between variety and spray rate on necrosis in pot trial #2 (Table 3.6). In Russet Burbank all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control. The 6.25mg/L treatment had significantly higher necrosis scores than all other 2,4-D treatments. The 12.5mg/L treatment was not significantly different to the 25mg/L, 50mg/L and 100mg/L treatments, but had a significantly mean higher necrosis score than the 200mg/L and 400mg/L treatments. The 25mg/L through to 200mg/L treatments were not significantly different from each other. The 400mg/L treatment had a significantly lower mean necrosis score than all other treatments. In Desiree all treatments resulted in significantly higher toxin tolerance (lower necrosis scores) than the control, except the 6.25mg/L treatment, which gave equivalent toxin tolerance to the control. The 100mg/L treatment was not significantly different to the 400mg/L treatment, and had significantly lower necrosis scores than all other treatments, except the 200mg/L treatment, which it was significantly higher than.

The 400mg/L treatment was not significantly different to the 25mg/L treatment, and the 12.5mg/L, 25mg/L and 50mg/L treatments were not significantly different to each other. The 6.25mg/L 12.5mg/L and 50mg/L treatments were not significantly different to each other. (Table 3.6)

3.4.4.3 Field trial

As in the pot trials, there was a significant interaction between spray rate and variety on necrosis. In both varieties all treatments resulted in significantly higher toxin tolerance (lower mean necrosis scores) than the control treatment (Table 3.6). In Russet Burbank the 1.625mg/L treatment had significantly higher mean necrosis scores than the 6.25mg/L, 100mg/L, and 400mg/L treatments. The 25mg/L treatment had significantly lower mean necrosis scores than all other treatments. In Desiree the 1.625mg/L treatment had significantly higher mean necrosis scores than the 6.25mg/L treatment, and was not significantly different from the 25mg/L, 100mg/L and 400mg/L treatment. The 6.25mg/L treatment had significantly lower necrosis scores than the 1.625mg/L and 100mg/L treatments, but was not significantly different to the 25mg/L and 400mg/L treatments. (Table 3.6)

3.4.5 2,4-D Quantification

3.4.5.1 Pot trial #2

Russet Burbank had a significantly greater amount of 2,4-D in tubers at harvest than Desiree. In both varieties there is a trend towards increase levels of 2,4-D in tubers at harvest with increasing application rates. In both Russet Burbank and Desiree the control has the lowest levels of 2,4-D (Figure 3.2). In Desiree the 50mg/L treatment had the lowest level of 2,4-D of all the treatments, but otherwise there is an increasing level of 2,4-D with increase application rate until the highest rate, 400mg/L, which resulted in slightly less 2,4-D than the 200mg/L treatment. In Russet Burbank the 6.25mg/L treatment had the lowest level of all the treatments, the 12.5mg/L, 25mgL and 50mg/L treatments have similar levels, and the 100mg/L and 200mg/L treatment have similar levels. As in Desiree, the Russet Burbank 400mg/L treatment is lower than the 200mg/L treatment.

The 100mg/L and 200mg/L treatments resulted in 2,4-D levels above the Australian MRL of 100ng/L of tuber fresh weight (Commonwealth of Australia 2011). This data lacks replication and as such has only been analysed statistically for varietal differences.

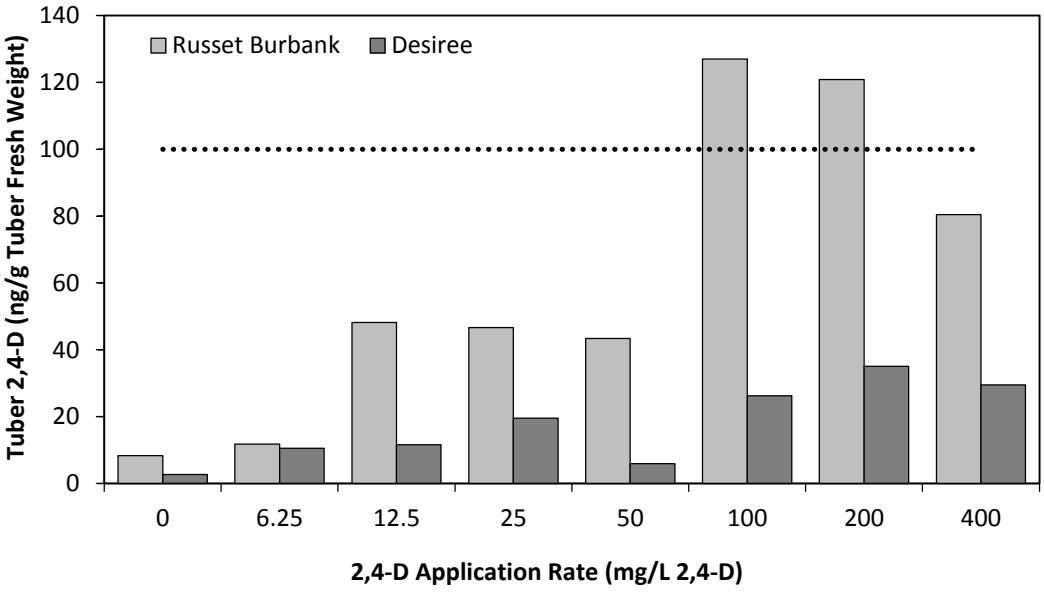

Figure 3.2 Field trial #2 The quantification of 2,4-D in tubers at harvest, from plants treated with 2,4-D foliar sprays applied at rates to the foliage of Russet Burbank and Desiree plants. Dotted line indicates the maximum residue limit (MRL) for 2,4-D in potatoes at harvest.

3.5 Discussion

3.5.1 Much lower rates than studied previously may control disease

The combined disease and tuber sensitivity data obtained in this study suggest that much lower rates of 2,4-D than were applied in prior studies (McIntosh *et al.* 1981; Tegg *et al.* 2008) may be as effective in controlling common scab. Initial trials (McIntosh *et al.* 1981) focused on an application rate of 200mg/L, which greatly reduced scab in glasshouse trials. In field trials, rates of both 100mg/L and 200mg/L of 3,5-dichlorophenoxyacetic (3,5-D) were found to be similarly effective in suppressing disease, but no effect on disease was found from 50mg/L applications (McIntosh *et al.* 1982). Later research (Tegg *et al.* 2008) found that 50mg/L applications of 2,4-D resulted in the same increased suppression of thaxtomin A toxicity in tubers as had been achieved by 200mg/L applications. Waterer (2010) found that much higher rates also suppressed disease, with a rate of approximately 647mg/L and 1294mg/L reducing common scab by similar amounts. The research presented in this chapter suggests that even lower rates of 2,4-D could be effective in controlling disease, with a trend towards as little as 1.625mg/L 2,4-D reducing disease cover in comparison to the control treatment in the variety Russet Burbank in the field trial. In pot trials, 6.25mg/L 2,4-D resulted in similar disease cover to that achieved by the previously published effective rates of 100mg/L and 200mg/L (McIntosh *et al.* 1981; 1982; Tegg *et al.* 2008).

That lower rates of 2,4-D appear to be able to control common scab disease is important due to the herbicidal properties of 2,4-D. The phytotoxic effects of 2,4-D (as well as the previously mentioned reduced effectiveness in field trials) meant that McIntosh and colleagues (1981) abandoned it as a possible commercial control method. The lowest label rate for 2,4-D as a herbicide is approximately 680mg/L for use on lawns (Nufarm Australia Limited 2011). The rates tested in prior published trials (McIntosh *et al.* 1981; 1982 ; Tegg *et al.* 2008; Waterer 2010), particularly when applied multiple times, resulted in near herbicidal levels of 2,4-D being applied to the plant over a period of 2-3 weeks during tuber development. This resulted in reduced yield, increased tuber number, and increased tuber deformity. Data presented in both this chapter and chapter 2 suggest that multiple sprays to “top up” tubers with 2,4-D are unnecessary, and that single sprays provide effective disease

control. However, The minimal amount of 2,4-D required for disease control is in contrast with the amount of material required by other chemicals that have been shown to suppress common scab. 2,5-dibromobenzoic acid (2,5-DBB) has been demonstrated to control common scab with minimal phytotoxic effects (McIntosh *et al.* 1988). Like 2,4-D, it appears to suppress the disease by reducing the sensitivity of the tuber to thaxtomin (Tegg *et al.* 2012). However, results obtained by Tegg *et al*. (2012) suggest that higher rates of 2,5-DBB are required to achieve similar disease control as 2,4-D.

3.5.2 Induced toxin tolerance threshold reached by low rates of applied auxin

That very low rates of 2,4-D may be as effective in controlling scab as previously published higher rates is further strengthened by the toxin tolerance of the harvested tubers. The foliar application of 2,4-D resulted in increased toxin tolerance of tubers in all three trials. This was also observed in the trials presented in chapter 2. Treatments as low as 1.625mg/L in the field trial and 6.25mg/L in the pot trials resulted in increased toxin tolerance, suggesting that very low levels of 2,4-D are required within the tuber for some degree of induced resistance. Quantification of the 2,4-D within tubers at harvest in pot trial #2 shows that, while much higher application rates resulted in higher 2,4-D levels within the tuber, the tolerance of these tubers to the toxin isn't increased. This suggests that there is a maximum level of toxin tolerance that can be induced by 2,4-D, and that this is reached by the foliar application of 2,4-D at rates much lower than had been previously tested. Application rates as low as 12.5mg/L provide sufficient 2,4-D to the tuber to induce a level of tolerance similar to that reached by 200mg/L, as used in previous studies (McIntosh *et al.* 1981; Tegg *et al.* 2008).

Quantification of 2,4-D from one pot trial found that applications of 100mg/L and 200mg/L to Russet Burbank plants resulted in levels of 2,4-D in the tubers at harvest above the MRL for potato in Australia, of 100ng 2,4-D/g tuber fresh weight (Commonwealth of Australia 2011). This is consistent with previous research (Tegg *et al.* 2008), which found rates as low as 50mg/L resulted in tuber 2,4-D at six weeks after treatment of twice the MRL. This suggests that these rates result in too much material being translocated to the tuber for it to be metabolised to consistently safe

levels before harvest. Toxin tolerance data suggest that this level of 2,4-D within the tuber is also unnecessary, with much lower amounts of tuber 2,4-D decreasing tuber toxin sensitivity to a similar level. The highest application rate treatment in the field trial was found have lower levels of 2,4-D in tubers at harvest than lower rates, which may be the result of this high rate damaging the plant and reducing translocation.

Levels of 2,4-D within Desiree tubers were significantly lower than in Russet Burbank tubers. While there is no published data on varietal differences in translocation of 2,4-D, carbohydrate flow, photosynthetic activity, growth rate and IAA levels, all of these influence the rate of translocation of phenoxyacetic acids (Burrell 1982). Varietal differences in these plant processes, or differences in plant development at the time of application may explain the varietal effect on tuber 2,4-D levels.

3.5.3 Lesion depth reduced by 2,4-D treatments

While only statistically significant in one pot trial, this study further suggests that 2,4-D can reduce lesion depth as well as lesion cover. This was found in previous studies that measured lesion depth (Tegg *et al.* 2008), but strong evidence of a reduction in lesion depth was not found in the trials presented in chapter 2. While a reduction in disease cover is a more visually impressive result, a reduction in lesion depth may be a more useful outcome. Common scab does not significantly reduce crop yield (Loria *et al.* 1997), but rather decreases quality and increases processing costs and waste. Deep lesions, a common symptom in Australia (Wilson *et al.* 1999) may still be present after normal steam peeling processes, while superficial lesions may not require any extra processing (Wilson *et al.* 2009). Therefore, a control that reduced lesion depth sufficiently so that lesions were removed by initial peeling, yet didn't significantly decrease lesion cover, may be of use in processing potato crops.

3.5.4 Evidence for reduction in disease incidence

Previous studies on auxin-induced resistance to common scab have focused on a reduction in disease severity (McIntosh *et al.* 1981; 1982; Tegg *et al.* 2008). The low levels of disease in the pot trials allowed for the calculation of disease incidence, measured by the proportion of diseased tubers, and in pot trial #2, the number of lesions per diseased tuber. The reduction in disease incidence from 2,4-D treatments at rates as low as 6.25mg/L was observed in both pot trials. This is the first demonstration that foliar 2,4-D treatments may also reduce disease incidence within a crop, as well as disease severity on individual tubers. In both trials Desiree had a higher proportion of diseased tubers than Russet Burbank, which is consistent with previous research that finds Desiree, while more tolerant to the toxin, to be more susceptible to the disease (Wilson *et al.* 2010). The proportion of tubers with any lesions within a crop can be used as a determinant of crop health and quality, and as such reducing disease incidence could be beneficial. This is particularly important in seed tuber certification (Wilson *et al.* 1999).

3.5.5 Tuber slice assays

Desiree tubers were treated with twice the concentration of thaxtomin A compared to Russet Burbank, as it has a naturally greater thaxtomin tolerance (Tegg & Wilson 2010), and therefore direct comparisons between the tolerance of the two varieties cannot be made. In both pot trials, all 2,4-D treatments, including the lowest rate of 6.25mg/L, suppressed thaxtomin toxicity in Russet Burbank, while in Desiree all 2,4-D treatments except the lowest rate had this effect. Lower rates therefore seem to be more effective in suppressing thaxtomin toxicity in Russet Burbank than in Desiree. This is consistent with the 2,4-D quantification data, which showed that more 2,4-D is translocated to tubers in Russet Burbank than in Desiree, and suggests that higher application rates of 2,4-D are required in Desiree to provide sufficient 2,4-D to tubers. However, all treatments in the field trial, including the lowest 1.625mg/L treatment resulted in increased toxin tolerance in both varieties, and in fact this reduction was more pronounced in Desiree than in Russet Burbank, despite Russet Burbank tubers having higher 2,4-D levels.

3.5.6 Lower rates may reduce phytotoxic effect

While there were no significant effects on yield or tuber number in any trials, the trend seen in the pot trials of decreasing total tuber mass, and in pot trial #2 of decreasing mass per tuber and increasing mean number of tubers with increasing rate is consistent with previously published results. McIntosh *et al*. (1981; 1982) found that rates of 2,4-D and 3,5-D which were effective in controlling common scab resulted in phytotoxic effects on the plant, observing an increased number of tubers, reduced tuber mass, and increased deformity. Tegg *et al*. (2008) observed similar phytotoxic effects from equivalent rates of 2,4-D. Waterer (2010), using a rate of approximately 647mg/L, the label rate for 2,4-D use on potatoes in the USA, and more than triple of that used by McIntosh *et al*. (1981) and Tegg *et al*. (2008) resulted in a decrease in tuber size and an increase in tuber number, and rates double this also resulted in a decrease in yield. However, the effects varied with season and variety, and 2,4-D was considered to have little impact on yield at the 647mg/L rate, but did decrease yield at higher rates (Waterer 2010). The phytotoxic effects of 2,4-D on potatoes therefore appear to increase with application rate, but are negligible with very low rates used in this study that can still suppress common scab.

3.5.7 Treatments may control other pathogens

The correlation between powdery scab and common scab disease severity that was observed in field trial #1 of chapter 2 (Figure 2.2), repeated in this field trial. There was no significant effect found from 2,4-D on powdery scab disease severity, however in Russet Burbank there was a trend towards 2,4-D treated plants having less powdery scab disease cover than the control. While there are no directly repeated trials that have provided data for powdery scab severity (powdery scab disease was negligible in field trial #2 of chapter 2) this repeated finding suggests that there is an interaction between the development of the two diseases and that control of common scab by 2,4-D may have the subsequent effect of reducing powdery scab, that 2,4-D treatments also act to reduce powdery scab, or possibly a combination of both of these.

As discussed in chapter 2, this is in contrast to Waterer (2010), 2,4-D decreased common scab disease but increased powdery scab disease (at rates higher than presented in this study). Waterer suggested this increase in powdery scab to be an opportunistic invasion by the powdery scab pathogen as a result of less competition at pathogen entry sites created by the reduction in common scab disease. There is no published evidence that 2,4-D treatments reduce the extent of colonisation of the tuber surface by the common scab pathogen, rather, the levels of 2,4-D present in the tuber after treatment have been shown to have no effect on pathogen growth or survival (McIntosh *et al.* 1981; Tegg *et al.* 2008).

Martínez-Noël (2001) observed that IAA treatments in potatoes decreased the severity of late blight, and found that the pathogen, *Phytophthora infestans*, was inhibited by the auxin, at and below rates at which 2,4-D has been shown to not be inhibitory to *Streptomyces scabies* (Tegg *et al.* 2008). This suggests at the applied rates, 2,4-D treatments may be directly inhibitory to other potato pathogens.

3.6 Conclusion

Based on toxin tolerance, disease and agronomic data obtained from the three trials detailed in this chapter, one application of 2,4-D applied at a rate as low as 25mg/L and sprayed until runoff, may be effective in reducing common scab disease of potato without phytotoxic effects.

Chapter 4: Evaluation of 2,4-D seed tuber treatments and intergenerational carryover of 2,4-D in seed tubers for control of common scab in potato

4.1 Abstract

Chapters 2 and 3 of this thesis demonstrated that very low and very early foliar treatments of 2,4-D provided control against common scab of potato, building on earlier research that had found it to be an effective control, but with undesirable phytotoxic effects. This chapter further builds on the work presented in chapter 2, which demonstrated that foliar applications of 2,4-D prior to tuber initiation still resulted in the translocation of 2,4-D to tubers as they develop and thus provided disease control. This study aimed to determine if treatments of the seed tuber would similarly provide 2,4-D to developing tubers; to provide a preliminary study into the use of 2,4-D as a seed tuber treatment for the control of 2,4-D; and to determine the effects of 2,4-D carryover from foliar applications of 2,4-D to seed tuber crops. The data suggested that 2,4-D seed tuber treatments did provide 2,4-D to developing tubers and therefore controlled disease, and that 2,4-D carryover similarly resulted in 2,4-D being translocated to developing tubers. This study suggests that the treatment of both seed tuber crops and seed tubers with 2,4-D may be an effective, novel application method for the control of common scab.

4.2 Introduction

Common scab is an economically important potato disease caused by pathogenic *Streptomyces* spp. (Loria *et al.* 2006). The control of common scab is primarily achieved through a combination of management strategies, including targeted irrigation (Lapwood *et al.* 1973) and the delayed planting (Waterer 2002a) of disease free seed tubers of resistant varieties (Wilson *et al.* 2009). Seed tuber treatments are used to a lesser extent , with mancozeb and cement dusting used infrequently in Australia (Wilson *et al.* 1999). While seed tuber borne inoculum was long considered to be an important factor in disease (Pavlista 1996), a number of studies have found that seed borne inoculum has no significant effect, and that environmental factors are more important (Lapwood 1972; Adams & Hide 1981; Pavlista 1996). However, others have found it to be as important as soil-borne inoculum (Wilson *et al.* 1999; Wang & Lazarovits 2005). The relative importance of seed tuber and soil borne inoculum on disease severity is likely to be dependent on local factors such as soil type, the pathogen population, climate and agronomic practice (Wilson *et al.* 1999).

The majority of seed tuber treatments against common scab work through direct action against the pathogen either on the seed tuber surface, thus reducing seed tuber borne inoculum, or within the immediate soil surrounding the seed tuber once it has been planted. Seed tuber treatments that have been demonstrated to be effective include fumigants such as mustard meal (Al-Mughrabi 2010), biological controls such as antibiotic producing or competition introducing bacteria (Beauséjour *et al.* 2003; Al-Mughrabi 2010) and phages (McKenna *et al.* 2001), chitosan, an elicitor of plant defence mechanism with fungicidal properties (Beauséjour *et al.* 2003) and other biocides such as pentachloronitrobenzene (PCNB), fluazinam, flusulfamide, fenpiclonil and macozeb (Davis *et al.* 1974; Hooker 1981; Wilson *et al.* 1999).

When applied to potato foliage, 2,4-D is translocated to tubers (Burrell 1982) and has been be an effective control of common scab when applied as foliar treatments (McIntosh *et al.* 1981; 1982; Loria *et al.* 2006; Tegg *et al.* 2008; Waterer 2010). Tegg (2008) determined that 2,4-D suppressed the toxicity of thaxtomin A, a phytotoxin produced by the common scab pathogen (King *et al.* 1989), essential to its pathogenicity (King *et al.* 1991; Loria *et al.* 1995; Goyer *et al.* 1998; Kers *et al.* 2005). Further research (Chapter 2) demonstrated that foliar 2,4-D treatments applied

soon after tuber emergence were more effective in controlling common scab than treatments applied near tuber initiation, as had previously been tested. This suggested early sprays provided protection to tubers prior to their formation and development through the phase of infection susceptibility. However, early spray treatments are restricted by the requirement to achieve full, or near full emergence of the crop, to ensure most or all plants received the protective treatment. The success of these early treatments and issues surrounding emergence delays led to the hypothesis that plants could be treated prior to emergence, in the form of a seed tuber treatment.

Chapters 2 and 3 of this thesis show 2,4-D is present at low levels in harvested tubers following foliar treatment of the growing crop. Carryover of 2,4-D in vegetative propagules into a subsequent crop could have detrimental effects on crop performance if present in high enough concentration. Alternatively, residual 2,4-D may also provide protection of the subsequent crop from infection by the common scab pathogen.

This chapter outlines results from a preliminary study on a) the use of 2,4-D as a seed tuber treatment to determine if 2,4-D applied to seed tubers can effectively control common scab and to determine the most effective application method and rate, and to b) determine if foliar treatment of prior seed tuber crops with 2,4-D provides sufficient carryover of 2,4-D to give control in tubers grown from the resultant seed tubers.

4.3 Materials and Methods

4.3.1 Inoculum for pot trials

Pathogenic *Streptomyces scabies* strain G#20, initially isolated from a common scab infected tuber harvested from north-west Tasmanian in 1990, was used to produce inoculum for the pot trials. The isolate was grown on 10mL ISP2 (Shirling & Gottlieb 1966) agar slopes (10g/L malt extract, 4g/L yeast extract, 4g/L glucose, 12g/L agar, pH 7.3) until sporulation. Colonised agar slopes were then aseptically transferred to a sterilised mixture of 120g vermiculite and 500mL SAY solution (20g/L sucrose, 1.2g/L *L*-asparagine, 0.6g/L K_2HPO_4, 10g/L yeast extract, pH 7.2) (Labruyère 1971). The inoculum was incubated in the dark at 24ºC until profuse sporulation was observed at around 14 days.

Approximately 1L of colonised vermiculite inoculum was added to 25L of potting mix containing sand, peat and composted pine bark at a ratio of 10:10:80, at pH 6.0, premixed with Osmocote 16:3.5:10 N:P:K resin coated fertiliser (Scotts Australia Pty Ltd.) at the rate of 6kg/m^3, and thoroughly mixed with a cement mixer. The inoculum containing potting mix was then used to fill plastic planter bags of 5L capacity (200 x 200mm, Botany Horticultural, Queensland, Australia).

4.3.2 Planting material

A week prior to planting, potato tubers were removed from cold storage, where they had been stored since harvesting approximately 6 months before and were cut into approximately 15g pieces and left to suberise before planting.

Cultivars Russet Burbank (with moderately resistance to common scab) and Desiree (moderate susceptibility) were used in all trials. Tubers used in the 2,4-D treatment pot trial and field trial were disease-free mini tubers from glasshouse grown tissue culture plantlets and visually disease-free certified seed tubers respectively. In the 2,4-D carryover pot trial, tubers were visually disease-free tubers harvested from a field trial in the 2009/10 season (section 2.3.5.2) that had received various rates of 2,4-D applied at 20DAE.

4.3.3 Preparation of 2,4-D treatments

4.3.3.1 Treated tuber pot trial

2,4-D solutions were prepared by dissolving 400mg of crystalline 2,4-D (Sigma Aldrich, St. Louis, USA) in approximately 10mL of 70% ethanol over heat. This solution was then mixed with 1L of warm water to produce a 400mg/L solution. 750mL of this 400mg/L solution was retained, and the other 250mL was added to 750mL of warm water to produce a 100mg/L solution. This dilution series was continued until a 6.25mg/L solution was produced, giving four 2,4-D concentrations. A control solution of warm water only was used. Warm water was used for all dilutions to assist in keeping the 2,4-D in solution. Tween-80 was added at a rate of 0.5g/L to all 2,4-D and control treatments as a wetting agent.

4.3.3.2 Treated tuber field trial

The source of 2,4-D in this trial was the commercial herbicide Amicide 625 (Nufarm Ltd, Victoria, Australia) with 625 g/L 2,4-D present as dimethylamine and diethanolamine salts. The treatment concentrations were adjusted to ensure that an equivalent amount of the active 2,4-D molecule was present compared with that used in the pot trials. Three treatment rates were applied: 400mg/L, 50mg/L equivalence, and a control spray containing no 2,4-D. Two treatment methods were used dipping and spraying. All treatments were applied on the date of planting.

To prepare the 2,4-D solutions, 8.8mL Amicide 625 was dissolved in 1L of water, with 0.5g of Tween-80 added as a wetting agent. This 8.8mL/L Amicide solution was then mixed with 9L of water within a container to make a 880µL/L solution. This was determined to be the equivalent of a 400mg/L 2,4-D solution. This was repeated using 1.1mL of Amicide 625 to create a 50mg/L solution. The control solution was water and Tween-80.

4.3.4 Tuber treatment experiments

The ability of 2,4-D applied directly to seed tubers to provide protection against common scab in the subsequent crop was assessed in one pot trial and one field trial.

4.3.4.1 Pot trial

The trial was planted on 20th December, 2010. Plants emerged on 1st January 2011. The varieties were the same as used in pot trial #1. There were five dipping treatments of varying concentrations of 2,4-D: 400mg/L, 100mg/L, 25mg/L, 6.25mg/L 2,4-D and a water control treatment with no 2,4-D. Each treatment was applied to five tubers of each variety, and one tuber was planted per pot (50 pots in total), with pots arranged in a completely randomised block design. Plants were maintained as per the daughter tuber pot trial.

The dipping treatments (400mg/L, 100mg/L, 25mg/L, 6.25mg/L 2,4-D and water control) were applied on the day of planting. Seed tubers were immersed completely in the dipping solution for 30 minutes. Tubers were then removed and laid in a single layer to air dry for 15 minutes before planting. All pots were hand-watered throughout the duration of the trial, ensuring that the potting soil dried between watering events to maintain a suitable environment for disease (Lapwood & Hering 1970). No other pesticides were applied. Plants were grown outside on a concrete slab and subject to natural weather events and temperatures. At approximately 50 DAE an additional 1L of a combination of inoculated vermiculite and potting mix at a ratio of 1:2 by volume was added to each pot. Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 25th May, 2011. Tubers were stored at 4ºC in plastic netted bags for a maximum of 2 weeks before assessment.

Soil was brushed from tubers, which were then assessed for common scab disease severity using the methods described in section 2.3.6. Tuber number and fresh weight were measured and any tuber disfigurements were noted. Sensitivity to thaxtomin A was assessed in two tubers from each pot using the tuber slice assay

(Tegg *et al.* 2008). Tubers without obvious lesions were chosen and assayed following the method described in section 2.3.7.

4.3.4.2 Treated tuber field trial

The trial site was located upon *Forester Lodge*, the property of Mr. Wilson Geale at Waterhouse in North East Tasmania. The soil was predominately sandy. The immediate area had been sown with a trial potato crop in the previous season (Field Trial #2 in section 2.3.5.2), and a commercial processing potato crop in the season prior to that. The surrounding area had been sown with an oil seed crop in the previous season and a lucerne feed crop in the trial season. The trial was planted on 27th October, 2010. Two potato varieties were used, Russet Burbank and Desiree. The trial was arranged in a randomised split plot design, with subplots consisting of five plants of the same variety, and plots consisting of two subplots, one of each variety. To ease planting the subplots were not randomised within each plot. The treatments were replicated four times.

In the field trial two different application methods for treating seed tubers with 2,4-D were assessed, dipping and spraying. For the spraying treatment seed tubers were spread on the ground in a single layer and the visible side was sprayed, using a handheld sprayer, with the 2,4-D solution until the seed tubers were fully covered with the solution. The seed tubers were air-dried for 10 minutes before planting. For the dipping treatment the seed tubers were fully immersed in the 2,4-D solution for 20 minutes. The seed tubers were then removed and laid in a single layer and air-dried for 5 minutes before planting.

The average emergence date was estimated to be 20th November 2010. The trial was watered by centre pivot irrigation and hand weeded of potato volunteers and other weeds when required. After senescence the trial was sprayed with a desiccant (Reglone®, Syngenta Crop Protection, UK) as per industry standards, and then harvested mechanically on 4th April, 2011. All the tubers of each variety were collected and combined from the five plants within each plot. Tubers were stored at 4ºC for a maximum of one month prior to assessment.

Before assessment the tubers were washed to remove soil and then weighed to determine yield for each plot (the combined tubers from five plants). Tubers were assessed for common scab severity (DCS and LDS) and powdery scab severity (DCS) using the methods described in section 2.3.7. Tuber thaxtomin sensitivity was assessed using the method described in section 2.3.7, modified so that tubers were taken from replicates 1 and 2 of the trial, and two tubers were assayed from each replicate (one tuber per Petri dish), with eight filter paper discs distributed over the slices from each tuber. A total of 32 filter paper discs were spread over four tubers from two replicates in four Petri dishes, for each treatment.

4.3.5 Carryover effect of 2,4-D into tubers

A single pot trial assessed the capacity of residual 2,4-D within tubers from foliar application of the previous crop to protect against common scab.

The pot trial was planted on 20th December, 2010. Daughter tubers from potato plants that had been treated once with one of four different rates of 2,4-D: 400mg/L, 100mg/L, 50mg/L, 0mg/L 2,4-D were planted in pots. No further treatments were applied to the tubers. Each treatment of each variety was replicated five times (40 pots in total), with pots arranged in a completely randomised block design. Plants emerged on 30th December, 2010. Plants were maintained as per the treated tuber pot trial.

Plants were grown to senescence, after which the pots were left without water for a fortnight before the tubers were harvested on 25th May, 2011. Tubers were stored at 4ºC in plastic netted bags for a maximum of 2 weeks before assessment.

Disease, yield and toxin sensitivity assessments were as per the treated tuber pot trial.

4.3.6 Data analysis

Data was analysed in Genstat 12.1 (VSN International Ltd., 2009). Multivariate Analysis of Variance was used to determine significant effects from, and interactions between treatment factors. Probabilities less than 0.05 were considered to be significant and Fischer's least significant difference (LSD) test was used for comparison of treatment means. Linear Regressions were used to examine the relationship between powdery scab and common scab severity.

4.4 Results

4.4.1 Disease control

4.4.1.1 Treated tuber pot trial

There was no significant effect found from treatment rate on DCS, mean number of lesions or LSD. Desiree had a significantly higher DCS and LDS than Russet Burbank. There was low emergence within the control treatment (Table 4.1).

4.4.1.2 Treated tuber field trial

Both rates of 2,4-D treatment resulted in lower DCS than the control treatment (Table 4.2). The 400mg/L treatment resulted in lower LDS than the control treatment. The 50mg/L treatment was not significantly different from either the 400mg/L or control treatments. There was no effect found from application method on DCS or LDS. Russet Burbank had lower DCS and LDS than Desiree.

There was a strong positive linear relationship between common scab and powdery scab DCS (Figure 4.1). However, there was no significant effect found from the seed tuber treatments on powdery scab disease cover (Table 4.3). Russet Burbank had a lower DCS of powdery scab than Desiree.

Table 4.1 Pot Trial Treated Tubers The effect of 2,4-D seed tuber treatments applied as a dip at various rates to Russet Burbank and Desiree seed tubers prior to planting, on common scab disease severity measured as a) the area of tuber surface covered by scab lesions on tubers with any visible lesions measured using the Disease Cover Score (section 2.3.6), b) derived percentage cover (ANOVA not performed on this data), c) the mean number of lesions per tuber, and d) the depth of lesions measured using the Lesion Depth Score (section 2.3.6). There was no significant effect found from rate. There was a significant effect found from variety on DCS ($p = 0.001$, SED = 0.1237, LSD = 0.2488) and LDS ($p < 0.001$, SED = 0.1117, LSD = 0.2248). There were no significant interactions between factors. Treatments with the same letter in the same row and variable are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Rate (mg/L 2,4-D)	Disease Cover Score (DCS) (and Percentage Cover (%))			Mean Number of Lesions			Mean Lesion Depth Score (LDS)		
	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
0	0.76 (1.62%)	1.25 (4.01%)	1.00	1.78	1.94	1.86	0.93	1.10	1.01
6.25	1.10 (3.18%)	1.46 (7.2%)	1.28	1.9	4	2.95	1.11	1.56	1.34
25	1.30 (6.42%)	1.64 (7.92)	1.47	3.9	3.05	3.47	1.22	1.86	1.54
100	0.89 (2.71%)	1.31 (5.24%)	1.10	2.17	2.42	2.29	1.07	1.42	1.25
400	1.10 (3.78%)	1.65 (8.07%)	1.37	2.99	5.06	4.02	1.13	1.91	1.52
Mean (Variety)	1.06 a	1.48 b		2.63	3.44		1.11 a	1.62 b	

Table 4.2 Field Trial The effect of 2,4-D seed tuber treatments applied as a dip or spray at various rates to Russet Burbank and Desiree seed tubers prior to planting, on common scab disease severity measured as a) the area of tuber surface covered by scab lesions on tubers with any visible lesions measured using the Disease Cover Score (section 2.3.6), b) derived percentage cover (ANOVA not performed on this data) and c) the depth of lesions measured using the Lesion Depth Score (section 2.3.6). There was no significant effect found from method of application. There was a significant effect found from rate on DCS ($p < 0.001$, SED = 0.131, LSD = 0.278) and LDS ($p = 0.043$, SED = 0.104, LSD = 0.221). There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.115, LSD = 0.242) and LDS ($p < 0.001$, SED = 0.089, LSD = 0.186). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Application Method	Rate (mg/L 2,4-D)	Disease Cover Score (DCS) (and Percentage Cover (%))			Lesion Depth Score		
		Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean
Dip	**0**	1.38 (4.73%)	2.46 (15.06%)	1.92	1.4	2.2	1.8
	50	0.93 (2.35%)	2.37 (13.36%)	1.65	1.2	2.2	1.7
	400	0.90 (2.47%)	1.86 (8.96%)	1.38	1.2	1.8	1.5
Spray	**0**	1.57 (5.92%)	2.33 (14.35%)	1.95	1.3	2.3	1.8
	50	0.86 (1.76%)	1.72 (7.99%)	1.29	1.1	2.0	1.5
	400	0.78 (1.68%)	1.62 (6.53%)	1.2	1.1	1.8	1.5
Mean (Rate)	**0**	1.48	2.39	1.93 a	1.3	2.3	1.8 a
	50	0.9	2.04	1.47 b	1.1	2.1	1.6 ab
	400	0.84	1.74	1.29 b	1.2	1.8	1.5 b
	Mean (Variety)	1.07 a	2.06 b		1.2 a	2.0 b	

Table 4.3 Field Trial The effect of 2,4-D seed tuber treatments applied as a dip or spray at various rates to Russet Burbank and Desiree seed tubers prior to planting, on powdery scab disease severity measured a) as the area of tuber surface covered by scab lesions on tubers with any visible lesions measured using the Disease Cover Score (section 2.3.6) and b) derived percentage cover (ANOVA not performed on this data). There was no significant found from method of application or rate. There was a significant effect found from variety on DCS ($p < 0.001$, SED = 0.097, LSD = 0.204). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Application Method	Rate (mg/L 2,4-D)	Powdery Scab Disease Cover Score (DCS) (and Percentage Cover (%))		
		Russet Burbank	Desiree	Mean
Dip	**0**	1.52 (6.18%)	2.46 (13.95%)	1.99
	50	1.41 (5.58%)	2.22 (13.11%)	1.83
	400	1.43 (5.42%)	2.23 (13.31%)	1.81
Spray	**0**	1.83 (8.1%)	2.51 (14.75%)	2.17
	50	1.26 (4.25%)	2.26 (12.53%)	1.91
	400	1.32 (5.08%)	2.49 (15.67%)	1.76
Mean (Rate)	**0**	1.68	2.49	2.08
	50	1.34	2.24	1.79
	400	1.38	2.36	1.87
	Mean (Variety)	1.46 a	2.36 b	

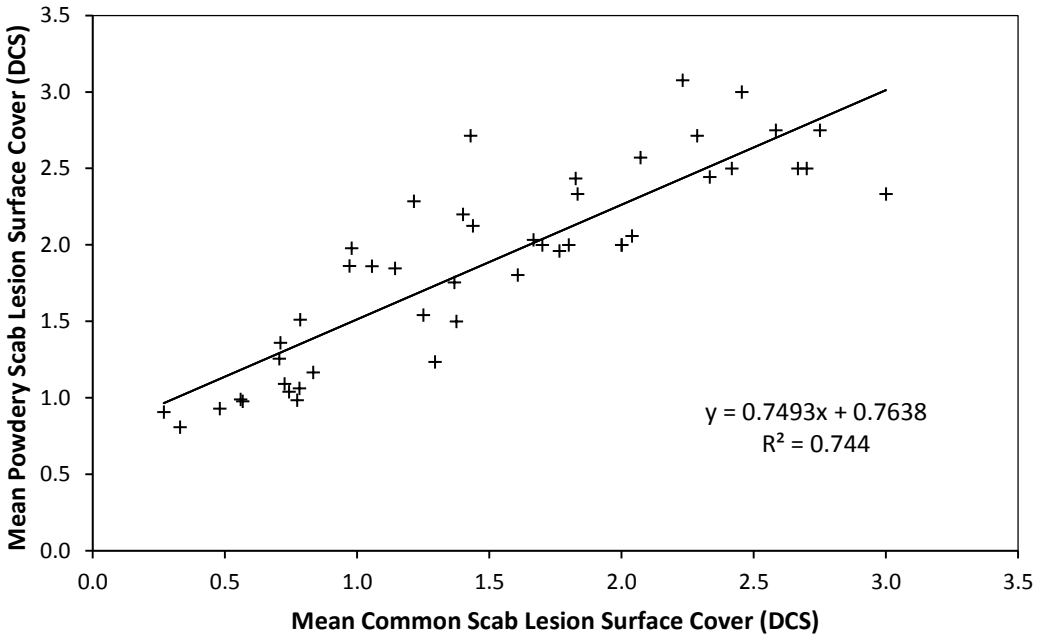

Figure 4.1 Field trial Linear regression between the mean surface coverage of common scab and powdery scab, on tubers harvested from Russet Burbank and Desiree plants treated with 2,4-D at various rates. $R^2 = 0.744$. Two outlying points were removed from analysis.

4.4.1.3 Carryover pot trial

There was a significant interaction found between spray rate and variety on DCS. In Russet Burbank the 400mg/L treatment resulted in significantly lower DCS than the control treatment. The 50mg/L treatment and the control were not significantly different from each other. In Desiree the 50mg/L treatment had significantly lower DCS than the 400mg/L treatment and the control, which were not significantly different from each other. There was also a significant interaction between spray rate and variety on mean number of lesions per tuber. In Russet Burbank the 400mg/L treatment resulted in significantly fewer lesions than the control treatment, and the 50mg/L and control treatments were not significantly different from each other. In Desiree the 50mg/L treatment resulted in significantly fewer lesions than the 400mg/L treatment. Neither 2,4-D treatment was significantly different from the control treatment. There was no significant effect found from spray rate on LDS, but Russet Burbank had significantly shallower lesions than Desiree. (Table 4.4)

Table 4.4 Pot Trial Carryover The effect on common scab severity in tubers grown from seed tubers harvested from Russet Burbank and Desiree plants that had previously been treated with foliar applications of various rates of 2,4-D, measured as a) the area of tuber surface covered by scab lesions on tubers with any visible lesions measured using the Disease Cover Score (section 2.3.6), b) derived percentage cover (ANOVA not performed on this data), c) the mean number of lesions per tuber, and d) the depth of lesions measured using the Lesion Depth Score (section 2.3.6). There was a significant interaction found between rate and variety on DCS ($p < 0.001$, SED = 0.131, LSD = 0.278) and mean number of lesions ($p = 0.043$, SED = 0.104, LSD = 0.221).

Spray Rate (mg/L 2,4-D)	Disease Cover Score (DCS) (and Percentage Cover (%))			Mean Number of Lesions			Mean Lesion Depth Score (LDS)		
	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
0	1.53 a (6.58%)	1.71 a (8.99%)	1.62	5.4 a	3.7 ab	4.6	1.35	2.07	1.71
50	1.18 ab (6.03%)	0.82 b (2.05%)	1.00	4.3 ab	1.8 b	3.1	1.20	1.22	1.21
400	0.80 b (1.93%)	1.86 a (9.17%)	1.33	2.0 b	5.3 a	3.6	1.33	1.97	1.65
Mean (Variety)	1.17	1.46		3.9	3.6		1.29 a	1.75 b	

4.4.2 Agronomic effects

4.4.2.1 Treated tuber pot trial

There was a significant effect found from treatment rate on total mass, mean mass per tuber, and mean number of tubers (Table 4.5). The 6.25mg/L treatment had the highest total mass. The 100mg/L and 400mg/L treatments were not significantly different from any other 2,4-D treatment. All treatments had greater total mass, mean mass per tuber and mean number of tubers than the control treatment. However, there was very low emergence within the control treatments compared to the 2,4-D treatments (Table 4.5), and as such the agronomic data is not reliable. Desiree was significantly greater for all factors.

4.4.2.2 Treated tuber field trial

There was no significant effect found from the seed tuber treatments on mean total mass, mean mass per tuber, or mean number of tubers (Table 4.6). However, there was a trend towards a lower total and per tuber mass, and number of tubers in both 2,4-D dipping treatments compared to the control treatment, and in the 400mg/L spray treatment compared to both the 50mg/L spray treatment and the control, which were similar. Russet Burbank had a significantly greater total tuber mass and number of tubers than Desiree. There was no significant effect found from variety in mean mass per tuber.

4.4.2.3 Carryover pot trial

There was a significant interaction between spray rate and variety on total tuber mass (Table 4.7). In Russet Burbank the 50mg/L treatment resulted in significantly lower total tuber mass than the control treatment. The 400mg/L treatment was not significantly different from either the 50mg/L treatment or the control. In Desiree the 400mg/L treatment resulted in significantly lower total tuber mass than both the 50mg/L treatment and the control, which were not significantly different from each other. There was no significant effect found from the 2,4-D treatments on either mean mass per tuber or number of tubers. There was no significant effect found from variety on total tuber mass or number of tubers. Desiree had significantly higher mean mass per tuber than Russet Burbank.

Table 4.5 Pot Trial Treated Tubers The effect of 2,4-D seed tuber treatments applied as a dip at various rates to Russet Burbank and Desiree seed tubers prior to planting, on mean total tuber mass, mean mass per tuber, mean number of tubers and percentage emergence (ANOVA not performed on this data). There was a significant effect found from rate on total mass ($p < 0.001$, SED = 6.12, LSD = 12.3), mean mass per tuber ($p < 0.001$, SED = 0.987, LSD = 1.984) and mean number of tubers ($p = 0.019$, SED = 1.359, LSD = 2.707). There was a significant effect found from variety on total mass ($p < 0.001$, SED = 3.33, LSD = 6.7), mean mass per tuber ($p < 0.001$, SED = 0.537, LSD = 1.08) and mean number of tubers ($p = 0.023$, SED = 0.74, LSD = 1.473). There were no significant interactions between variables. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Rate (mg/L 2,4-D)	Total Mass (g)			Mean Mass per Tuber (g)			Mean Number of Tubers			Emergence (%)	
	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree
0	3.2	4.3	3.7 a	1.3	1.0	1.2 a	0.6	1.2	0.9 a	20	20
6.25	29.3	51.1	40.2 c	4.1	6.9	5.5 b	4.5	6.8	5.7 b	70	90
25	19.3	32.0	25.7 b	3.0	7.0	5.0 b	4.0	4.9	4.5 b	50	100
100	13.7	42.7	28.2 bc	2.5	8.2	5.3 b	2.4	5.9	4.2 b	50	100
400	26.5	40.5	33.5 bc	4.5	8.7	6.6 b	3.4	4.1	3.8 b	60	80
Mean (Variety)	20.1 a	37.5 b		3.3 a	7.0 b		3.2 a	5.0 b			

Table 4.6 Field Trial Treated Tubers The effect of 2,4-D seed tuber treatments applied as a dip or spray at various rates to Russet Burbank and Desiree seed tubers prior to planting, mean total tuber mass, mean mass per tuber, and mean number of tubers. There was no significant found from method of application or rate. There was a significant effect found from variety on total mass ($p < 0.001$, SED = 211.1, LSD = 653.6) and number of tubers ($p < 0.001$, SED = 2.75, LSD = 5.77). There were no significant interactions between factors. Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's Least Significant Difference (LSD) test.

Method	Rate (mg/L 2,4-D)	Total Mass (g)			Mass per Tuber (g)			Number of Tubers		
		Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean	Russet Burbank	Desiree	Mean
Dip	**0**	4085	1564	2824	95.1	129.5	112.3	43.8	13	28.4
	50	3861	914	2388	87.4	96.4	91.9	45	7.8	26.4
	400	3620	836	2228	80.4	102.3	91.3	45.5	8	26.8
Spray	**0**	3951	1538	2744	87.4	94.9	91.1	47.2	13.3	30.2
	50	4161	1314	2738	87.8	92.4	90.1	48.2	13.5	30.9
	400	3230	652	1941	81.5	73.1	77.3	39.2	7.5	23.4
Mean (Rate)	**0**	4018	1551	2784	91.3	112.2	101.7	45.5	13.1	29.3
	50	4011	1114	2562	87.6	94.4	91.0	46.6	10.6	28.6
	400	3425	744	2085	80.9	87.7	84.3	42.4	7.8	25.1
Mean (Variety)		3818 a	1136 b		86.6	98.1		44.8 a	10.5 b	

Table 4.7 Pot Trial Carryover The effect from replanting tubers harvested from Russet Burbank and Desiree plants that had previously been treated with foliar applications of various rates of 2,4-D, on mean total tuber mass, mean mass per tuber, and mean number of tubers. There was a significant interaction found between rate and variety on total mass ($p = 0.001$, SED = 7.4, LSD = 16.15). There was a significant effect found from variety on mean mass per tuber ($p = 0.032$, SED = 1.909, LSD = 3.252). Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate (mg/L 2,4-D)	Total Mass (g)			Mass per Tuber (g)			Number		
	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)	Russet Burbank	Desiree	Mean (Rate)
0	59.0 a	101.8 a	80.4	10.7	13.9	12.3	5.2	7.4	6.3
50	39.4 b	104.9 a	72.1	7.6	14.2	10.9	5.6	8.2	6.9
400	77.4 ab	62.1 b	69.7	13.4	14.3	13.9	6.6	4.6	5.6
Mean (Variety)	89.6	58.6		10.5 a	14.1 b		5.8	6.7	

4.4.3 Tuber toxin tolerance

4.4.3.1 Treated tuber pot trial

There was a significant interaction found between spray rate and variety on mean necrosis score (Table 4.8). In Russet Burbank, the 6.25mg/L and 400mg/L treatments had significantly lower necrosis than the 25mg/L treatment. The 100mg/L treatment was not significantly different to any 2,4-D treatment. All treatments increased toxin suppression compared to the control. In Desiree, all treatments had similarly increased toxin suppression compared to the control.

Table 4.8 Pot Trial Treated Tubers The effect of 2,4-D seed tuber treatment on mean necrosis levels from the treatment of tuber slices with thaxtomin A. Tubers harvested from Russet Burbank and Desiree plants that had been grown from seed tubers treated with various rates of 2,4-D were tested for sensitivity to thaxtomin A. Necrosis was rated using the scale described in section 2.3.7. There was a significant interaction found between rate and variety ($p = 0.001$, SED = 0.0652, LSD = 0.1286). Treatments with the same letter, in the same column, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Rate (mg/L 2,4-D)	Mean Necrosis Score		
	Russet Burbank	Desiree	Mean (Rate)
0	1.72 a	1.94 a	1.83
6.25	1.23 c	1.18 b	1.21
25	1.45 b	1.18 b	1.31
100	1.28 bc	1.09 b	1.18
400	1.12 c	1.26 b	1.19
Mean (Variety)	1.32	1.26	

4.4.3.2 Treated tuber field trial

There was a significant effect found from application rate on mean necrosis score (Table 4.9). The 400mg/L treatment had significantly lower necrosis than both the 50mg/L treatment and the control treatment. The 50mg/L treatment also had significantly lower necrosis than the control treatment.

Table 4.9 Field Trial The effect of 2,4-D seed tuber treatment on mean necrosis levels from the treatment of tuber slices with thaxtomin A. Tubers harvested from Russet Burbank and Desiree plants that had been grown from seed tubers treated as dips and sprays with various rates of 2,4-D were tested for sensitivity to thaxtomin A. Necrosis was rated using the scale described in section 2.3.7. There was a significant effect found from rate ($p = 0.005$, SED = 0.0652, LSD = 0.3501) and variety ($p = 0.006$, SED = 0.1809, LSD = 0.4428). There was no significant effect found from application method. There were no significant interactions between factors. Treatments with the same letter, in the same column or row are not significantly different at $p = 0.05$ using Fischer's LSD test.

Application Method	Rate (mg/L 2,4-D)	Mean Necrosis Score		
		Russet Burbank	Desiree	Mean
Dip	**0**	2.56	1.58	2.07
	50	2.23	1.05	1.64
	400	1.64	1.03	1.34
Spray	**0**	2.48	1.41	1.95
	50	1.72	1.48	1.60
	400	1.28	0.84	0.06
Mean (Rate)	**0**	2.52	1.49	2.01 a
	50	1.98	1.27	1.62 b
	400	1.46	0.94	1.20 c
	Mean (Variety)	1.99 a	1.23 b	

4.4.3.3 Carryover pot trial

There was a significant effect found from spray rate on mean necrosis score (Table 4.10). Both 2,4-D treatments had a similarly increased toxin suppression compared to the control.

Table 4.10 Pot Trial Carryover The effect of 2,4-D foliar spray carryover on mean necrosis levels from the treatment of tubers slices with thaxtomin A. Tubers harvested from Russet Burbank and Desiree plants that had been treated with foliar applications of various rates of 2,4-D were replanted and the resultant tubers were tested for sensitivity to thaxtomin A. Necrosis was rated using the scale described in section 2.3.7. There was a significant effect found from rate ($p < 0.001$, SED = 0.0798, LSD = 0.1575) and variety ($p = 0.001$, SED = 0.0652, LSD = 0.1286). Treatments with the same letter, in the same column, or the same row and variable, are not significantly different at $p = 0.05$ using Fischer's LSD test.

Spray Rate (mg/L 2,4-D)	Mean Necrosis Score		
	Russet Burbank	Desiree	Mean (Rate)
0	2.19	1.97	2.08 a
50	1.31	1.17	1.24 b
400	1.30	1.02	1.16 b
Mean (Variety)	1.60 a	1.39 b	

4.5 Discussion

4.5.1 2,4-D applied as a seed tuber treatment is translocated to emerging tubers

This study suggests that treatment of the seed tubers with 2,4-D does control common scab in the resultant crop. Unlike most other seed tuber treatments, which reduce seed tuber borne inoculum through biocidal activity on the pathogen (Wilson *et al.* 1999; McKenna *et al.* 2001; Al-Mughrabi 2010), this seed tuber treatment likely acts in a similar way to 2,4-D foliar treatments, with material being translocated through the plant to the tuber, and suppressing thaxtomin toxicity, thereby controlling disease (McIntosh *et al.* 1981; Tegg *et al.* 2008; Waterer 2010). Tuber slice assays suggest that 2,4-D seed tuber treatments do result in material being translocated to daughter tubers, and corresponds with previous work that demonstrated that foliar treatments of 2,4-D shortly after emergence, and prior to tuber initiation, resulted in the translocation of 2,4-D to tubers as they are formed, thus providing protection against infection.

While the 2,4-D appears to have been translocated to daughter tubers from the treated seed tubers, it is unknown how the 2,4-D passed into the seed tuber. The seed tubers were sprouting at the time of treatment, and it is likely that 2,4-D would have been taken up by the tuber through these actively growing shoots, as it is taken in through foliage (McIntosh *et al.* 1981; Tegg *et al.* 2008; Waterer 2010). However, the 2,4-D may have also been absorbed through the skin of the tuber during the period of soaking. Burrell (1984) found that tuber discs absorbed sufficient 2,4-D through soaking to suppress thaxtomin toxicity. The treatment may also have stayed on the outside of the tuber as a coating, and was slowly absorbed by the plant. The rate at which the 2,4-D was translocated from the seed tuber to the daughter tuber is also unknown, but that disease control was achieved suggests that it was translocated to the tuber before or around the time of initiation.

4.5.2 Low levels of 2,4-D within tubers control disease in subsequent crop

It was unknown prior to this work whether the direct application of 2,4-D to the seed tuber would result in sufficient levels of material within the daughter tuber for disease control. The results of this study demonstrated that seed tubers soaked for 20 minutes with a 50mg/L solution of 2,4-D uptake sufficient material that is then translocated to daughter tubers at levels capable of controlling disease. Previous work had demonstrated that rates as low as 1.625mg/L of foliar applied 2,4-D resulted in toxin suppressive levels of 2,4-D within the tuber at harvest (Chapter 3). These results again suggest that only low levels of 2,4-D need to enter the plant (either as foliar sprays or through seed tuber treatment) for sufficient material to be translocated to daughter tubers to both control disease and suppress thaxtomin toxicity. Quantification of 2,4-D within both the treated seed tubers and the harvested tubers were unavailable in these trials due to the expensive and time-consuming nature of the quantification method, and the preliminary nature of this study.

4.5.3 2,4-D Seed tuber treatments are not biocidal

Wilson *et al*. (1999) found that the treatment with the biocides PCNB, fluazinam, flusulfamide, fenpiclonil and macozeb of seed tubers with visible lesions gave effective control, while the treatment of visibly clean seed tubers was not effective, suggesting that these chemicals control common scab through a reduction in seed tuber borne inoculum, but have little effect on the ability of the pathogen already present within the soil to induce disease on daughter tubers. Neither the application rates used in this study, nor the levels of 2,4-D present in the tubers from foliar treated plants affect the growth of *S. scabies* (McIntosh *et al.* 1981; Tegg *et al.* 2008). Therefore, tuber treatments of 2,4-D are unlikely to control disease through a reduction in inoculum levels either on the seed tuber, or in the surrounding soil, and control is likely a result of the suppression of thaxtomin toxicity.

4.5.4 2,4-D seed tuber treatments may promote emergence

Emergence was not negatively affected by the 2,4-D treatments; rather there was an observed reduction in emergence amongst the control treatment in the treated tuber pot trial. There is conflicting evidence on the role of auxin in tuber dormancy, and relatively little research into this area. Auxin has been widely considered to be an inhibitor of dormancy breaking, and may induce the production of ethylene, which in turn induces abscisic acid (ABA), which inhibits dormancy breaking (Suttle & Hultstrand 1994; Suttle 2003). However, auxin levels have been found to increase within potato tuber buds towards the end of dormancy (Sorce *et al.* 2000), and auxin response genes are upregulated in the potato tuber apical bud upon the release from dormancy (Faivre-Rampant *et al.* 2004). It has also been suggested that an increase of auxin may be involved in the early termination of dormancy through inducing early developmental processes (Sorce *et al.* 2009). Exogenous auxin has also been demonstrated to negate the yield reduction in plants grown from physiologically older seed tubers (Kumar & Knowles 1993).

The reduced emergence observed within the control treatment, compared to those treated with 2,4-D within the pot trial was an interesting result, and may be a result of this auxin induced dormancy breaking. The possible involvement of auxin in the induction of cell development, as suggested by Sorce *et al*. (2009), may indeed have resulted in the treated seed tubers emerging and becoming established more quickly than the control treatments, possibly reducing their susceptibility to pathogens or predators. Another possible explanation is that, while tubers were removed from cool storage a fortnight prior to planting, dormancy may not have been broken at the time of planting, and the 2,4-D induced dormancy breaking in the treated tubers. However, a large number of the control plants failed to emerge completely, rather than late in comparison to the treated plants. It is important to note that reduced emergence within the control was not seen in the similar field trial and has therefore not been replicated, and as such this observation may simply be random, rather than a treatment effect.

4.5.5 Carryover of 2,4-D may provide multi-generational disease control

As foliar treatments of 2,4-D result in thaxtomin suppressive levels of 2,4-D within tubers at harvest, it was possible that, if planted as seed tubers, this 2,4-D could be translocated to daughter tubers of the resultant crop providing multi-generational disease suppression. While the exact amount of 2,4-D within the tubers at planting was unknown, tubers were likely to contain between 10 and 100ng/g FW 2,4-D at harvest, based on the quantification of 2,4-D within tubers harvested from similar treatments (Figure 2.4). While these results are only from a single pot trial, they suggest that plants grown from tubers that contain residual 2,4-D from foliar treatments can have reduced disease severity. The data from all the trials suggests seed tuber 2,4-D treatment (or accumulation of 2,4-D within the seed tuber) could provide novel and efficient disease control. Wilson *et al*. (1999) demonstrated the importance of seed tuber borne inoculum in common scab. Foliar treatment of potato seed tuber crops with 2,4-D would have the combined effect of reducing disease on seed tubers through the suppression of thaxtomin toxicity and the prevention of infection, thus reducing seed tuber borne inoculum levels, and providing control against common scab from soil borne inoculum in the resulting crop. Low rates of foliar applied 2,4-D were not found to negatively affect yield, tuber number or shape, nor were phytotoxic effects observed in this study from tuber treatments or carryover 2,4-D, and as such treatments of seed tuber crops with 2,4-D would be unlikely to negatively affect the crop, or the seed tubers.

4.5.6 Conclusion

This study suggests that 2,4-D seed tuber treatments do result in the translocation of 2,4-D to tubers as they develop, and provide the disease control similar to foliar applications of 2,4-D. It also suggested that the carryover of 2,4-D in tubers harvested from crops treated with foliar applications of 2,4-D provided disease control to the next generation of tubers. Neither of these application methods negatively affected emergence or yield, and may provide a novel application method for the control of common scab by 2,4-D.

Chapter 5: Evaluation of genetic diversity within the *TXR1* homolog in thaxtomin resistant potato varieties

5.1 Abstract

In *Arabidopsis*, mutations within the gene *TXR1* resulted in increased resistance to the toxin produced by the potato common scab pathogen, thaxtomin, through its reduced uptake. We isolated and sequenced the homolog of *TXR1* in four somaclonal variants of Russet Burbank and the parent line to determine if there was variation within the gene that may explain the increased thaxtomin resistance phenotype exhibited by the variant lines. Ten unique alleles were found across the five lines, and compared with a previously published *TXR1* potato homolog and possible homolog from the recently published diploid potato genome. There were a higher number of unique alleles found in all the variant lines compared to the parent, and the number of alleles found suggests that the *TXR1* homolog may be duplicated across the genome. While no premature terminations were found, the increased level of mutation within the variants alleles may partly explain the thaxtomin resistant phenotype.

5.2 Introduction

Common scab, one of the most economically important diseases of potato worldwide, is caused by a number of pathogenic *Streptomyces* spp., with the most prevalent being *S. scabies* (Loria *et al.* 1997). Pathogenic *Streptomyces* spp. produce a number of phytotoxins called thaxtomins (King *et al.* 1989), and the production of these has been shown to be crucial to pathogenicity (Loria *et al.* 1995; Goyer *et al.* 1998; King *et al.* 2001; Kers *et al.* 2005). Thaxtomin A causes necrosis when applied directly to potato tubers in the absence of the pathogen (Lawrence *et al.* 1990). It causes the detachment, but not the rupturing, of the plasmalemma from the cell wall in mature potato tuber parenchyma cells (Goyer *et al.* 2000), and may inhibit biosynthesis or deposition of cellulose within the cell wall (Fry & Loria 2002).

A thaxtomin resistant *Arabidopsis thaliana* mutant was characterised by Scheible *et al*. (2003) and designated *txr1*. They determined there was a point mutation in the gene *TXR1*, where an Arg codon at amino acid 98 had been replaced with a stop codon leading to early termination of the gene. The mutant had an increased tolerance to thaxtomin A compared to the wild type. The phenotype was determined to be the result of reduced cellular uptake of thaxtomin A rather than a change in the metabolism of the toxin, which was similar in both the mutant and the wild type. The *txr1* mutant had retarded growth compared with the wildtype, which suggested that the native protein is required for normal growth. Scheible *et al*. (2003) proposed that the TXR1 protein has an involvement with cellular macromolecule transport, acting as a cytosolic regulator of a membrane protein rather than a component of a transport mechanism, and may be involved with thaxtomin uptake.

Homologs of *TXR1* have been found in many plants (Scheible *et al.* 2003), including potato (AJ Conner, AgResearch Ltd., New Zealand, pers. comm.), which suggests that it has a conserved function. The cDNA sequence of the potato homolog has been published as SGN-U294912 in the SGN database (Bombarely *et al.* 2011).

Somaclonal variants of the commercial cultivar Russet Burbank with enhanced resistance to common scab disease have been obtained in prior studies using a novel cell selection technique with thaxtomin A as a positive selection agent (Wilson *et al.* 2010). Several of these common scab resistance somaclonal variants possessed

enhanced tolerance to thaxtomin A when evaluated in a tuber slice toxin assay (Wilson *et al.* 2001).

Analogous to the thaxtomin A tolerant *Arabidopsis* txr1, mutations within the potato *TXR1* homolog in the somaclonal variants may be, at least partly, the cause of the observed thaxtomin resistance in these lines. In this chapter we compare the *TXR1* homolog nucleotide and amino acid sequences of four variants to the Russet Burbank parent.

5.3 Materials and Methods

5.3.1 Line selection and plant material

Four common scab resistant Russet Burbank variants that consistently showed diminished sensitivity to thaxtomin A in tuber slice assays (section 2.3.7) were used in this study. The variants were obtained by somatic cell selection from the parent cultivar Russet Burbank Vancouver clone (A184b; TC9-T4; TC9-M1) or from the variant TC9-T4 (TC9-T4*) in a second round selection, using thaxtomin A as a positive selection agent (Wilson *et al.* 2010).

The four variants and the Russet Burbank parent were maintained and propagated in tissue culture (Tegg *et al.* 2008), in a semi-solid potato propagation medium (0.8% agar, 3% sucrose, 0.05% casein hydrolysate, 0.004% ascorbic acid, pH 5.8) (Murashige & Skoog 1962).

5.3.2 DNA extraction

Leaf tissue samples (5g) were taken from the terminal ten leaves of 3-4 tissue cultured plantlets of each variant and the parent. DNA extraction from leaf samples was undertaken using the FastDNA DNA extraction kit (MP Biomedicals, OH, USA) following the manufacturer's instructions, using the FastPrep FP120 (Bio101, CA, USA). DNA samples were stored at -20ºC until used.

5.3.3 Amplification of TXR1 homolog DNA

An approximately 600bp fragment of the *TXR1* potato homolog gene was amplified by PCR using primers MAQ-3S (5'-ATG GCT GCA AAA ATT CTT G-3') and MAQ-2A (5'-TTA TTT TGT TTC TGG TTC-3') developed by Tegg (2006) from the cDNA sequence of the putative potato homolog (SGN-U294912) (Figure 5.1). Each 50µL reaction contained 25µL HotStartTaq Master Mix (Qiagen, CA, USA), giving a final concentration of 2.5 units/µL of HotStarTaq DNA polymerase, 1.5mM $MgCl_2$, and 0.2mM of each of the dNTPs, 1µM of each primer, 1µL of template DNA and 22µL sterile water. The PCR was performed on a Mastercycler Gradient (Eppendorf, Hamburg, Germany) with an initial denaturation of 95ºC for 15 minutes,

followed by 35 cycles of 94ºC for 1 minute, 42ºC for 30 seconds, and 72ºC for 1 minute, and a final extension at 72ºC for 10 minutes.

```
                    10        20        30        40        50        60        70
                ....|....|....|....|....|....|....|....|....|....|....|....|....|
AT3G59280  204 ATGGCTGGGAGACTACTTGCAAATTTGATTGTGATGGGTTCTGGGATCATTGGTC~GTGCTGTCTTTCAA 273
SGN-U29491 1   .......CA.A.A.T.....T.....A.....A.....C...TCT..~.....CAA.G...T.TG..... 69

                    80        90       100       110       120       130       140
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AT3G59280  274 GCCTATCGTCAAGCACTTG~CTAATGCGTCTAAATCTGGTGTTGCGCAGGAAGCAATGCAAAATGGAGTA 343
SGN-U29491 70  ..A........G...~...T.C.....C..G..GAA.........T..A......G....G...ATTAA. 138

                   150       160       170       180       190       200       210
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AT3G59280  344 CG~TCAAGCAGGGAAAGCCATCACTGA~GCAAGAGGCTAGGCAGATTCTTGGTGTAACCGAGAAGACCTC 412
SGN-U29491 139 A.A..T....~~~...~....G..G..A...~.....A..A..............C..A...G.TT.A.. 203

                   220       230       240       250       260       270       280
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AT3G59280  413 TTGGGAAGAGATATTACAGAAATATGACAAACTGTTTGAGAATAATGCGAA~AGCAGGGAGCTTTTACCT 482
SGN-U29491 204 A........A..CG.G.....G........CT........CGA.....T..G.A.~.....T........ 272

                   290       300       310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
AT3G59280  483 TCAATCTAAAGTTCATCGAGCCAAAGAATGTCTAGAAGTTGTGTACAGAAGCCAAGGCAACGGTACACCT 553
SGN-U29491 273 ......A..G..A...A....T.....G...T.G....AA..TC...~..C.T..A.A.C.A.A.~..AA 340

                   360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|...
AT3G59280  554 AGTTAAGACCTTACTCTTTTTGTTTTCCAGACTCAAAATTCTTGCTCTTAATG 607
SGN-U29491 341 .A.~....AAA.T~.G.GC.GA.G..~~..TG.~.T.~........GC..... 387
```

Figure 5.1 cDNA sequence from potato (*Solanum tuberosum)* (SGN-U294912) aligned to a section of the cDNA sequence (position 204 to 607) of the *TXR1* gene in *Arabidopsis thaliana* (AT3G59280). Dots indicate no change between sequences. This section of the *TXR1* sequence has a 70% sequence identity with the potato homolog.

5.3.4 Electrophoresis

Successful amplification of a product of approximately 600bp was confirmed by electrophoresis of 5µL aliquots of PCR product in 3% agarose gels in lithium borate buffer (0.2395g LiOH, 1.625g boric acid, 100µL SYBRsafe 1L deionised water, pH 8.5) at 250V for 20 minutes. After electrophoresis, gels were analysed under UV light using a Kodak EDAS290 digital camera with a 49mm ultraviolet lens (Kodak, CT, USA) and an Invitrogen Safe Imager Transluminator (Invitrogen, CA, USA). Images were viewed and edited using Kodak 1D LE 3.5 (Kodak, CT, USA).

5.3.5 Cloning of PCR product

Amplified DNA was cloned using the TOPO TA Cloning® Kit for Sequencing using One Shot® TOP10 Chemically Competent *E. coli* cells and pCR®4-TOPO® plasmid (Invitrogen, CA, USA) according to the manufacturer's instructions, except 6µL rather than 3µL of cloning reaction was added to each vial of cells. Cells were plated on LB agar (40g tryptone soy agar, 5g NaCl, 5g yeast extract, 1L distilled water) containing 100mg/L ampicillin for the selection of colonies, successfully transformed with the plasmid. Thirty-two colonies for each variant and the parent control were selected, transferred into 50µL of sterile water and stored at -20ºC.

5.3.6 Recovery and amplification of cloned PCR product

Cloned *TXR1* homolog sequences were amplified from each colony by PCR. Each 20µL reaction contained 0.125µL of the proof reading AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA), 2µL GeneAmp PCR AmpliTaq Gold Buffer 10x Buffer (Applied Biosystems, CA, USA) giving a final concentration of 15mM Tris-HCl (pH 8.0), and 50mM KCl, 3µL 25mM $MgCl_2$, 0.25µM each of the four dNTPs, 1µM each of the primers M13F (5'-GTA AAA CGA CGG CCA G-3') and M13R (5'-CAG GAA ACA GCT ATC AC-3'), 1µL of *E. coli* cells from the previous cloning step, and 6.875µL sterile water. The PCR was performed on a Mastercycler Gradient with an initial denaturation of 95ºC for 15 minutes followed by 35 cycles of 94ºC for 1 minute, 55ºC for 1 minute, and 72ºC for 1 minute, and a final extension at 72ºC for 10 minutes. Successful amplification of a product of approximately 720bp was confirmed by electrophoresis.

5.3.7 Preparation for DNA sequencing

Each PCR product was purified by QIAquick PCR Purification kit (Qiagen, CA, USA), and 2.5µL of purified template (approximately 100µg of DNA) then added to two wells of a 96 well plate, along with 7.2µL of RNase free water. Then 2.3µL of M13F (forward) primer (50µM) was added to the first well, and 2.3µL of M13R (reverse) primer (50µM) was added to the second.

DNA samples were then sent to the Australian Genome Research Facility (Melbourne, Australia) for sequencing using an AB 3730xl 96-Capillary DNA Analyser (Applied Biosystems, USA).

5.3.8 Analysis of sequenced DNA

Sequences were manually edited with ChromasPro, version 1.5 (© Technelysium 2003-2009). Sequences were manually aligned and predicted protein sequences were determined using BioEdit Sequence Alignment Editor 7.0.5.3 (Hall 1999). Allele sequences found only once were discarded as possible sequencing errors. The intron-exon boundaries were determined in comparison with data from Tegg (2006) and the published *TXR1* potato homolog (SGN-U294912). Predicted secondary protein structures were determined with Antheprot 2000 (Deleage *et al.* 2001) using the GOR method (Garnier *et al.* 1996).

5.4 Results

Ten unique allele sequences were identified in this study. Two alleles were identified in the Russet Burbank parent line at a 1:1 ratio and designated *TXR1*-a and *TXR1*-b. *TXR1*-a was present in all four variants, as was an allele identical to *TXR1*-b, except for a SNP within the second intron at position, (a G in *TXR1*-b, an A in all other alleles) designated *TXR1*-c. Two alleles (designated *TXR1*-d and *TXR1*-e) were found in all variants, but not the parent line. Of the ten alleles identified, five were found in A184b (*TXR1*-a, -c, -d, -e, -h), six in TC9-T4 (*TXR1*-a, -c, -d, -e, -f, -j) and TC9-T4* (*TXR1*-a, -c, -d, -e, -f, -g) and seven in TC9-M1 (*TXR1*-a, -c, -d, -e, -f, -g, -i). All alleles contain three exons and two introns. Polymorphisms were present in all exon and intron regions. Alleles ranged in size from 606-608nt long. See Figure 5.2 for the full nucleic acid sequence.

5.4.1 Allelic variation in the exons

There were no deletions found in any of the three exons in any allele. For all alleles, exon 1 was 91nt, exon 2 was 131nt and exon 3 was 123nt. There were two substitutions in exon 1, six substitutions in exon 2 and eight substitutions in exon 3.

5.4.2 Allelic variation in the introns

There was more variation in the introns than the exons, with 19 substitutions in intron 1 and 14 substitutions in intron 2. In intron 2, *TXR1*-j and *TXR1*-e had a single nt deletion at position 250 and *TXR1*-b, *TXR1*-c and *TXR1*-h had this deletion and an additional deletion at position 223, while other alleles had a substitution at position 250.

5.4.3 Exon-intron boundaries

In *TXR1*-e, found in all lines but the parent, the boundary between the second exon and second intron had a substitution from T to C, three base pairs into the intron. All other exon/intron boundaries were identical between alleles.

Figure 5.2 Allelic variants of the putative *S. tuberosum TXR1* homolog (*TXR1*-a through *TXR1*-j). Sequences consist of 3 exon and 2 intron regions separated by ~~~. Sequences are aligned to the homolog sequence identified in the diploid potato (The Potato Genome Sequencing Consortium 2011), between positions 360-881 of Superscaffold PGSC0003DMT400075996 (PCGS seq) and the putative *S. tuberosum* homolog cDNA sequence SGN-U29491.

```
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TXR1-c       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATAGTCAGGCATTGTCC 90
TXR1-d       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCATTTGTTCAAGCATATCGTCAGGCATTGTCC 90
TXR1-e       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
TXR1-f       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
TXR1-g       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCATTTGTTCAAGCATATCGTCAGGCATTGTCC 90
TXR1-h       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATAGTCAGGCATTGTCC 90
TXR1-i       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
TXR1-j       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
PGSC seq     ATGGCTGCAAAAATTATTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
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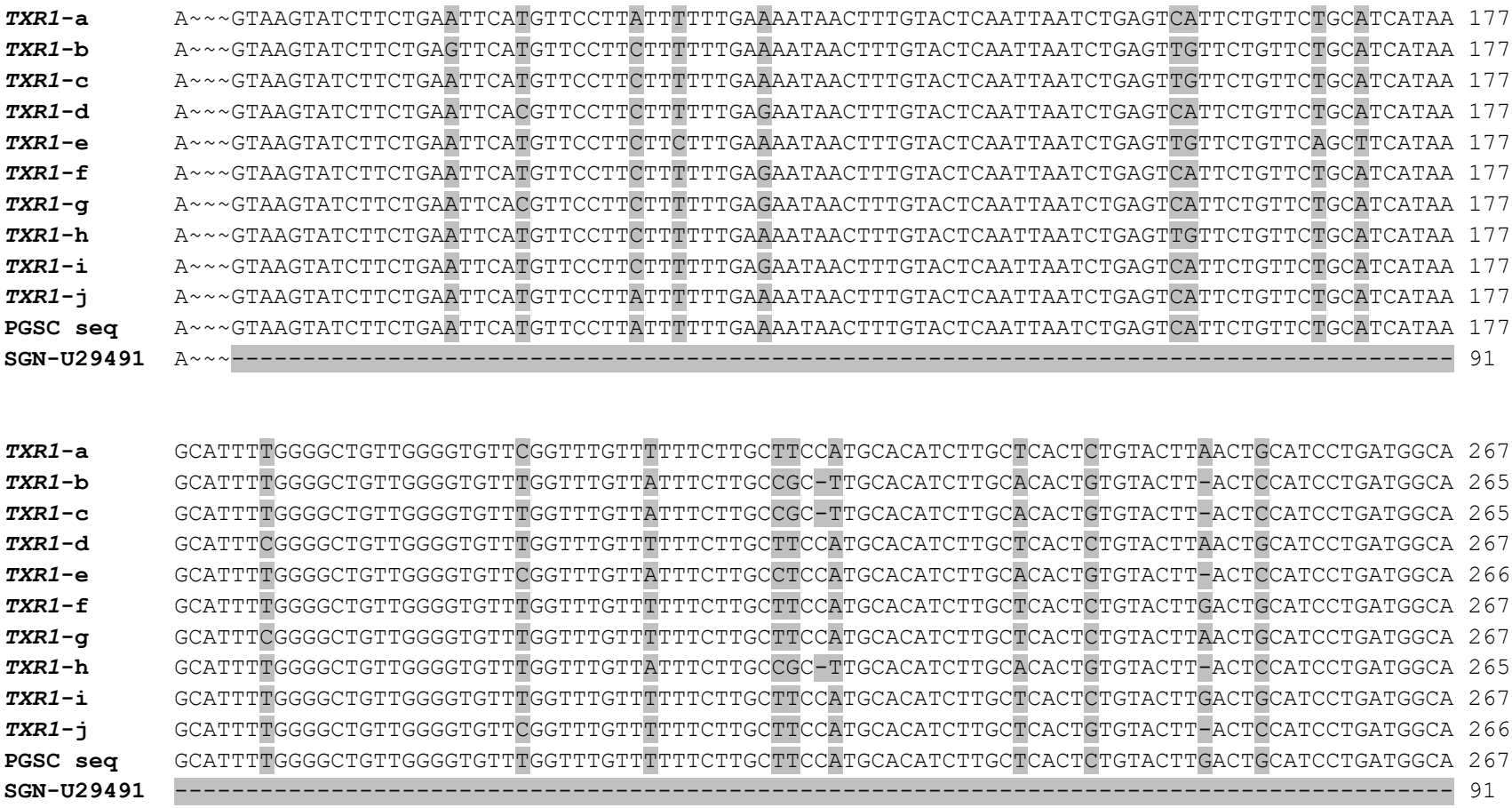
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TXR1-c A~~~GTAAGTATCTTCTGAATTCATGTTCCTTCTTTTTTGAAAATAACTTTGTACTCAATTAATCTGAGTTGTTCTGTTCTGCATCATAA 177
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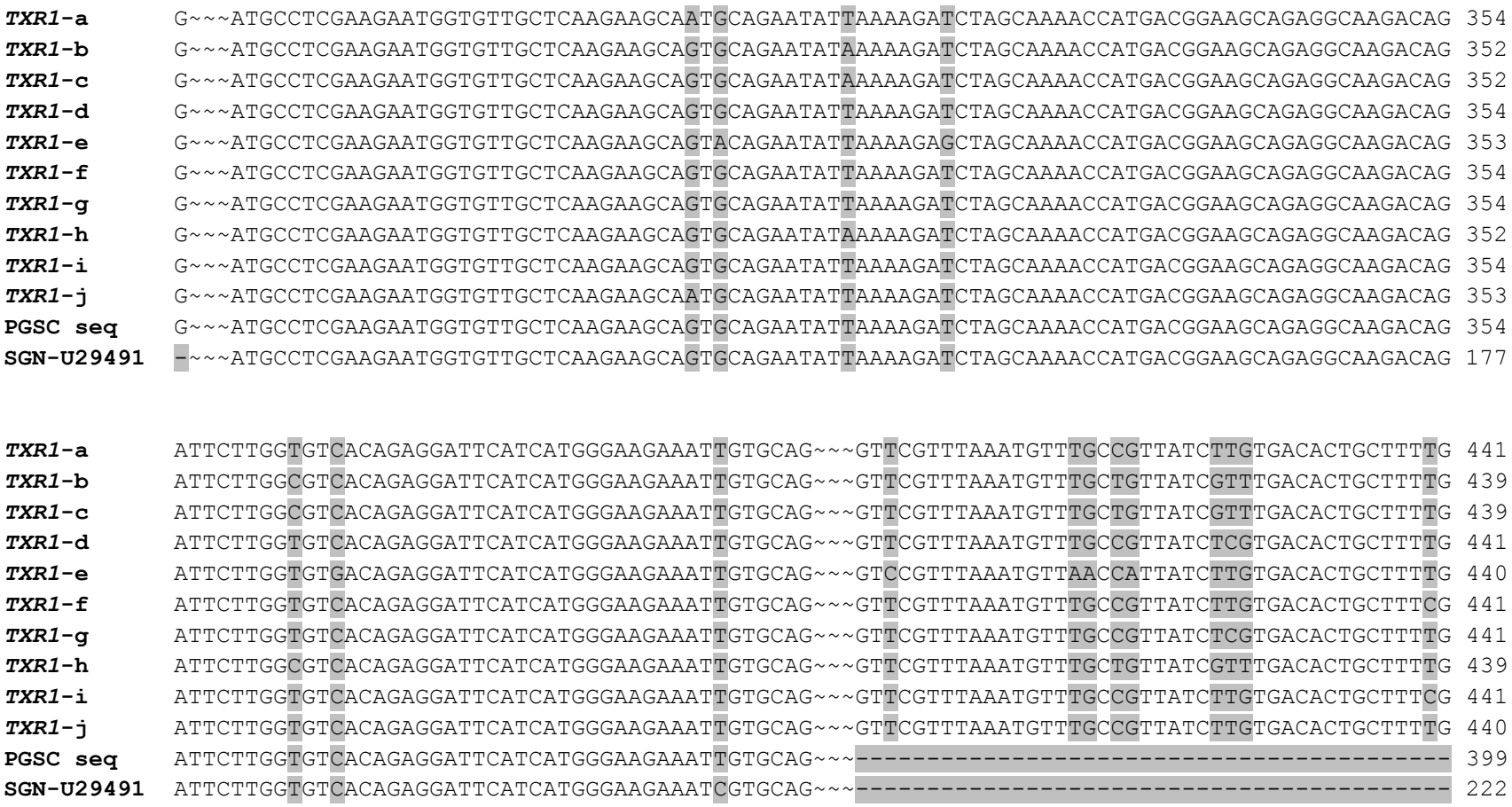
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SGN-U29491 TTTACCTTCAATCAAAGGTACATAGAGCTAAAGAGTGTTTGGAAGAAGTTCACAAACCTAAAGAACCAGAAACAAAATAA 345

5.4.4 Protein comparison

All alleles encode for a predicted protein 114 amino acids in length. There were no early terminations in any of the alleles identified. There were six instances where SNPs resulted in changes to the predicted amino acid sequence (one in exon 1, two in exon 2 and four in exon 3) while all other mutations were silent. There were two SNPs within the nucleotides coding for amino acid 106, which resulted in three different amino acids at this point: tyrosine, histidine, and leucine. The one substitution between *TXR1*-b and *TXR1*-c was in intron 1 and therefore did not result in a change in the predicted amino acid sequence. There were three differences in predicted amino acid sequence between *TXR1*-a and *TXR1*-b, the two alleles present in the parent line. The published SGN-U294912 allele had in the same amino acid sequence as *TXR1*-d and Allele 1 from Tegg (2006).

There were four main regions within the protein where differences may exist in the predicted secondary structures. The 5' end of the protein was identical in all alleles. Changes to amino acid 26 in alleles *TXR1*-b, *TXR1*-C and *TXR1*-h resulted in a sheet and coil within a helix, compared to a continued sheet in the other alleles. There are changes within the alleles *TXR1*-g, *TXR1*-h and *TXR1*-i, found in all variants but TC9-T4, near the 3' end of the predicted secondary structure. The predicted secondary structures of all alleles are presented in Figure 5.3.

Figure 5.3 Exons of the identified variants of the putative *S. tuberosum TXR1* homolog (*TXR1*-a through *TXR1*-j), predicted amino acid sequence, and predicted secondary protein structures (where | represents helix, = represents sheet, : represents turn, and blank space represents coil). Exons are separated by ~~~.

```
TXR1-a       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
TXR1-b       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATAGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  S  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  =        |
TXR1-c       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATAGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  S  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  =        |
TXR1-d       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCATTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
TXR1-e       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
TXR1-f       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
TXR1-g       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCATTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
TXR1-h       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATAGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  S  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  =        |
TXR1-i       ATGGCTGCAAAAATTCTTGCTAATTTAATTGTAATGGGCTCTTCTATATTGGCAAGGGCTTTTGTTCAAGCATATCGTCAGGCATTGTCC 90
             M  A  A  K  I  L  A  N  L  I  V  M  G  S  S  I  L  A  R  A  F  V  Q  A  Y  R  Q  A  L  S
             |  |  |  |  |  |  |  |  =  =  =  =              |  |  |  |  |  |  |  |  |  |  |
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             |  |  |  |  |  |  |  |  =  =  =              |  |  |  |  |  |  |  |  |  |  |
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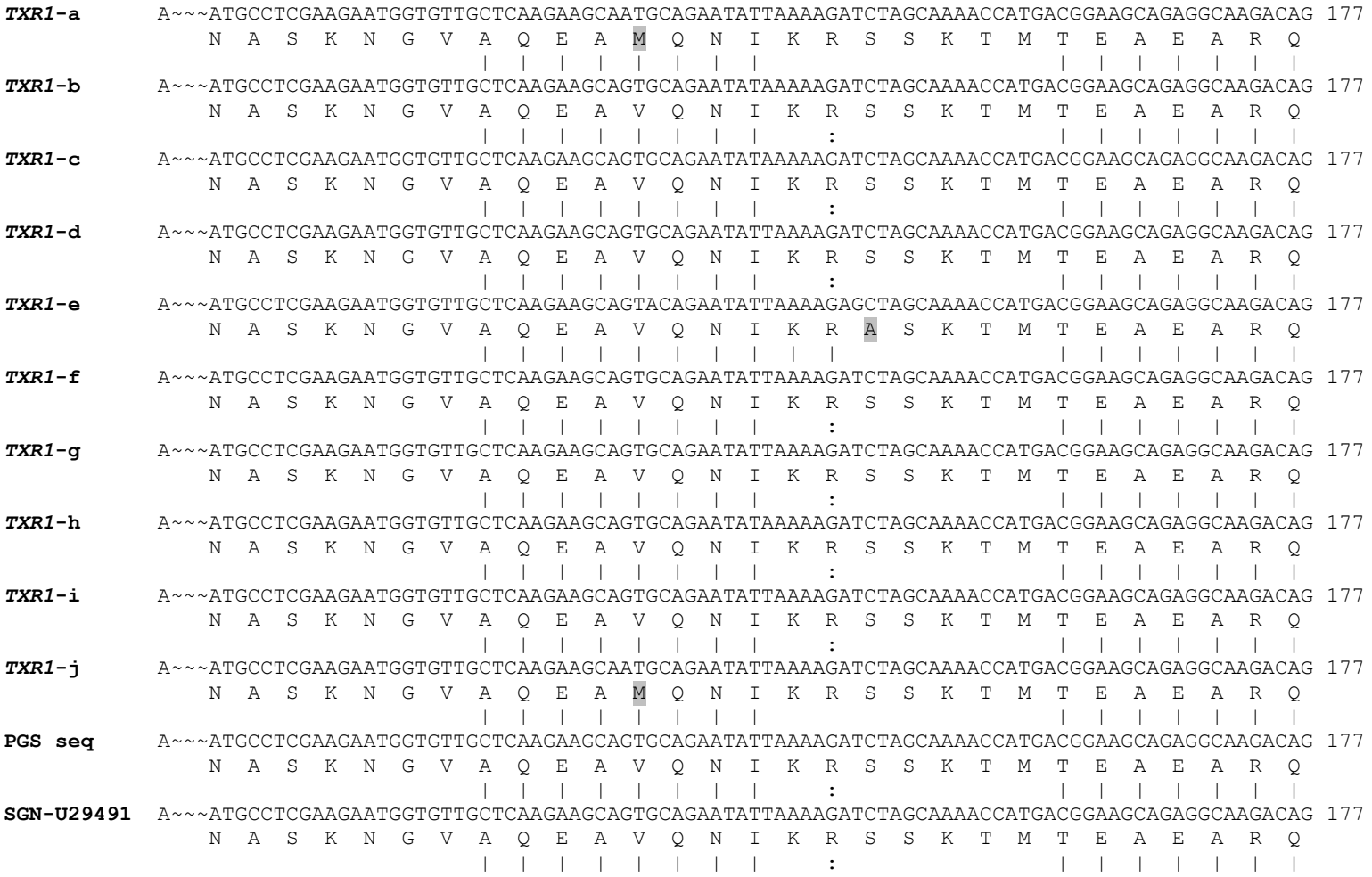
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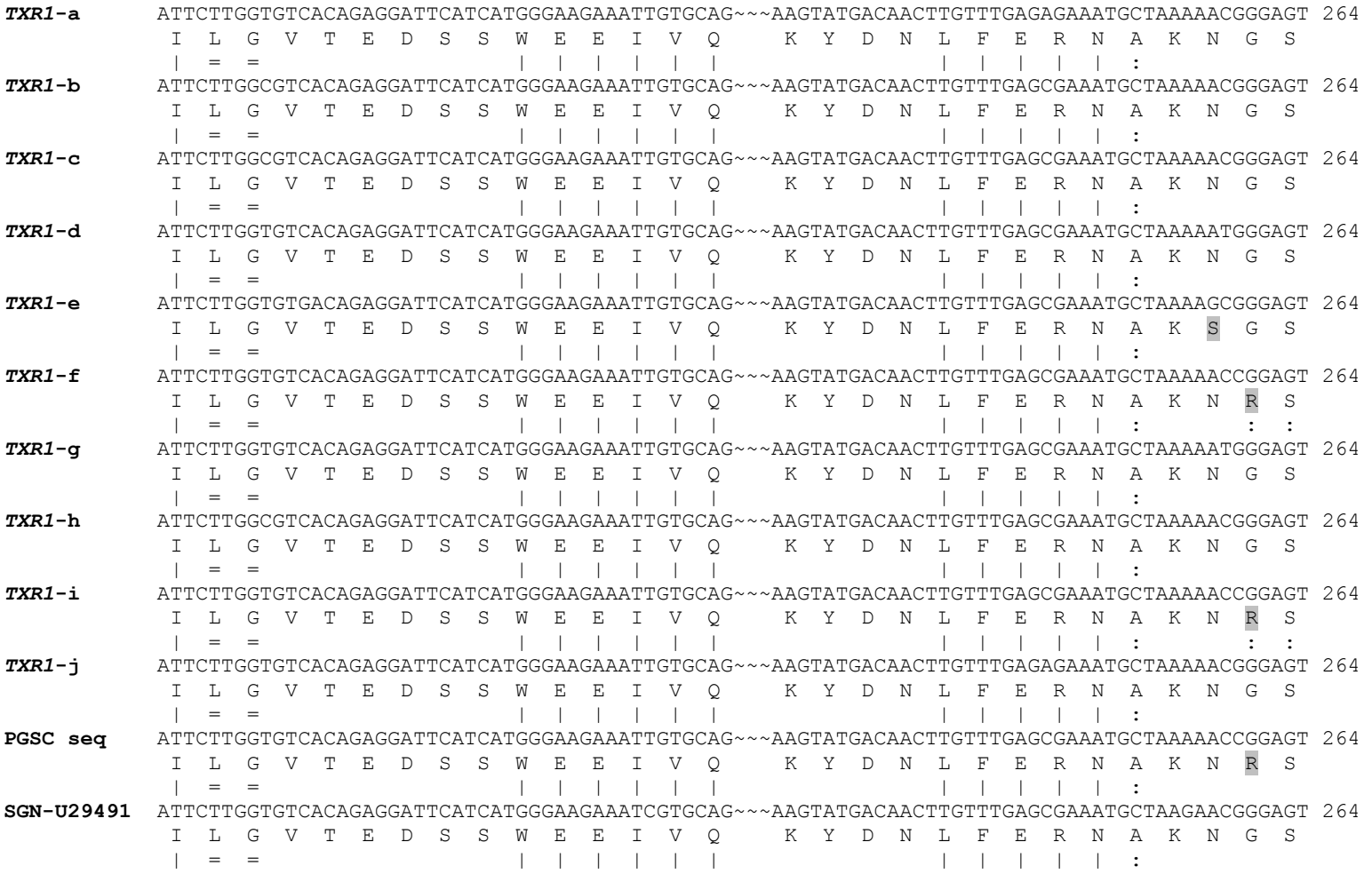
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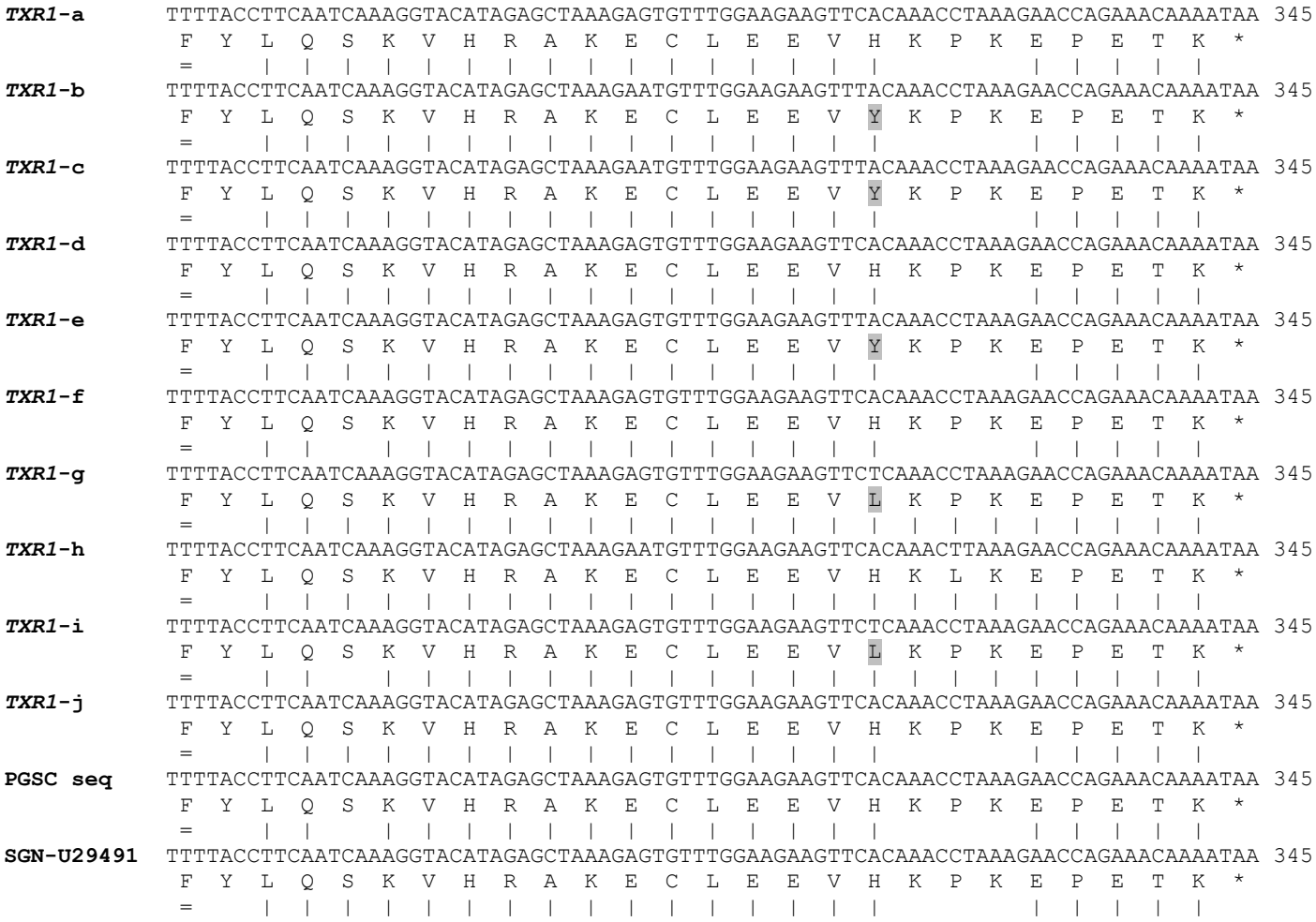
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F Y L Q S K V H R A K E C L E E V H K P K E P E T K *
TXR1-b TTTTACCTTCAATCAAAGGTACATAGAGCTAAAGAATGTTTGGAAGAAGTTTACAAACCTAAAGAACCAGAAACAAAATAA 345
F Y L Q S K V H R A K E C L E E V Y K P K E P E T K *
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F Y L Q S K V H R A K E C L E E V H K P K E P E T K *
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F Y L Q S K V H R A K E C L E E V L K P K E P E T K *
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F Y L Q S K V H R A K E C L E E V H K L K E P E T K *
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F Y L Q S K V H R A K E C L E E V H K P K E P E T K *

5.5 Discussion

5.5.1 Identification of previously unpublished unique alleles

The ten *TXR1* homolog alleles identified in this study have not previously been published. While the SGN-U294912 sequence was previously identified as a potato homolog of *TXR1* (A Conner, pers. comm.), it is a cDNA sequence and as such does not contain introns. Therefore the alleles identified in this study were named *TXR1*-a through -j as the first published alleles of the *TXR1* potato homolog.

5.5.2 Mutations within variant alleles may have effect on thaxtomin tolerance

The *TXR1* gene has an important cellular function that is proposed to involve regulation of a membrane transport protein. Independent studies have shown that silencing *TXR1* homologs in yeast (Scheible *et al.* 2003) and potato (A Conner, pers. comm.) is lethal, confirming the involvement of the gene in essential cellular processes. The diploid *txr1 Arabidopsis* mutant, which has one normal and one impaired *TXR1* allele, grows poorly in comparison to the wildtype (Scheible *et al.* 2003). Similarly, the four thaxtomin tolerant potato varieties examined in this study showed reduced growth compared to the parent cultivar (Wilson *et al.* 2010). Unlike the txr1 *Arabidopsis* mutant, none of the mutant *TXR1* homolog alleles had a premature stop codon. All variants had the two alleles found in the parent line (albeit with minor, silent mutations). There was an increased level of mutation within the alleles identified only in the variants, compared to the two identified in both the variants and the parent line, with a number of SNPs in the three exons that resulted in changes to the predicted amino acid sequence. These expressed mutations within the additional alleles found in the variants may have an effect on their growth characteristics as well as thaxtomin tolerance.

5.5.3 Mutations within introns of variants alleles may not be silent

All alleles were in full agreement with the GT-AG rule (Breathnach & Chambon 1981) at the intron exon boundaries, with GT at the 5' end and AG at the 3' end of both introns. However there was a SNP at position 3 of the second intron in allele *TXR1*-e, which was present in all variants but not the parent. Also, *TXR1*-b, found in the parent was identical to *TXR1*-c, found in the variants, except for a SNP at position 107, in the first intron. This SNP is only found in *TXR1*-b. There was a higher rate of SNPs within both intron regions than the exon regions, which is common in many organisms, including potato (Datir *et al.* 2012). Introns can regulate gene expression and contain promoter sequences (Morello & Breviario 2008) and therefore intron mutations are not necessarily benign. Therefore, these mutations within the intron could have an influence on the observed phenotype of the variants.

5.5.4 Insertion of intron into tetraploid potato

A BLAST search of the sequenced diploid potato genome (The Potato Genome Sequencing Consortium 2011) revealed a sequence of 1177 nucleotides (Superscaffold PGSC0003DMT400075996), with a 98.85% sequence identity (six substitutions and no SNPs) between positions 360-881 to *TXR1*-a. However, the diploid sequence lacks the second intron, which appears in both parent and variant sequences of the tetraploid Russet Burbank. This second intron is present in the diploid potato genome at another position. A BLAST search found a sequence with 100% sequence identity to the second intron of *TXR1*-a nearby in the genome (position 239-324 of Superscaffold PGSC0003DMT400076009). This suggests a duplication of this portion of this sequence into the *TXR1* homolog, and thus the creation of a new intron within the *TXR1* homolog in the tetraploid potato. As it is found in all ten alleles identified in this study, is in agreement with the GT-AG rule (Breathnach & Chambon 1981), and the silencing of this gene is lethal, this inserted intron does not appear to affect the translation of the gene, but may affect its regulation.

5.5.5 Changes in predicted secondary structure may affect function

There was a higher rate of polymorphism at the 3' end of the predicted amino acid sequence than the 5' end, suggesting that the 3' end of the protein in less conserved. The predicted secondary structure also suggests that the 5' end of the protein is more conserved. However, the predicted structure of the diploid potato homolog (Superscaffold PGSC0003DMT400075996) (The Potato Genome Sequencing Consortium 2011) had a slightly shorter sheet at the 5' end of the protein than all the identified alleles from the present study. This homolog resulted in a secondary structure most similar to *TXR1*-f, found in three of the variants. Scheible *et al*. (2003) postulated that the protein is probably not a permanent part of a transport mechanism, but as both the function and structure of the protein is unknown, we should be cautious in speculation on the importance or effect of changes at either end of the predicted secondary structure.

5.5.6 Suggestion of multiple copies of gene

Unlike *Arabidopsis*, potato is usually tetraploid, and can be highly heterozygous (Meyer *et al.* 1998). Between five and seven unique alleles were found in all the variants. As potato is a tetraploid species, this suggests that there may be two copies of the gene per genome, as a single copy should result in a maximum of four alleles. This was proposed by Tegg (2006), who characterised five unique alleles from both the same parent cultivar used in this study, and two different somaclonal variants. This study used high fidelity proof reading polymerase enzyme to recover and amplify the *TXR1* gene to minimise the potential of PCR induced error in sequences obtained. Furthermore, sequences obtained only once (from the 32 clones of each variant and parent) were discarded, as these were more likely to contain sequencing errors compared to allele sequences that were obtained multiple times.

5.5.7 Other mutations in variants are likely to influence phenotype

The somaclonal mutants were the result of a cell selection process using thaxtomin A as a positive selection agent. It is quite possible that *TXR1* is not the only gene responsible for thaxtomin resistance. In a separate study, evidence for significant genetic and epigenetic changers throughout the genome of potato variants was shown following cell selection with thaxtomin A (Dann & Wilson 2011). Thus mutations outside of *TRX1* would have occurred and could have influenced the thaxtomin resistance phenotype. Further research is currently being undertaken to sequence genomes of a selection of these variants and to compare this to the parent, which will provide greater details of the genetic differences induced by cell selection.

Chapter 6: General Discussion

6.1 Summary of Research

The research presented in this thesis has provided new and additional information to more accurately determine optimal timing, rates and number of applications required for effective control of common scab through foliar applications of 2,4-D, building on the research of McIntosh *et al*, (1981; 1982) Tegg *et al*. (2008; 2012); and Waterer (2010).

When examining similar rates to those that had previously been studied, it was found that foliar treatments of 2,4-D applied as early as five days after plant emergence provided effective control without significantly affecting yield or increasing tuber deformity. These very early treatments gave better and more reliable control than treatments that were timed to coincide with tuber initiation, and while being applied prior to the development of tubers, they still resulted in 2,4-D being translocated to tubers once tubers had developed. Additionally, only one 2,4-D application was required to ensure sufficient material translocated to the tuber to control disease for the entire period of susceptibility to infection by the common scab pathogen.

Much lower concentrations of 2,4-D than the near herbicidal levels that had previously been studied were equally effective in controlling disease. While rates as low as 1.625mg/L controlled disease and suppressed thaxtomin A toxicity in tubers at harvest, 25mg/L was consistently as effective as rates of up to 16 times this amount. Additionally, applications made at these times and rates resulted in levels of 2,4-D within tubers at harvest that were below the Australian MRL (Commonwealth of Australia 2011).

Disease control was achieved through both the treatment of seed tubers directly with 2,4-D, or the presence of 2,4-D within seed tubers following foliar treatment of seed tuber crops in the previous season. This research, while preliminary and requiring further study to optimise both the concentration of 2,4-D applied and the method of application, is both interesting and potentially valuable as an alternative means of applying and using 2,4-D as a disease suppressive agent.

6.2 Early treatments and translocation

Previous research had suggested that tubers are susceptible to pathogen entry through lenticels between 4-6 weeks after tuber initiation, during the period when stomata are transforming into lenticels but have yet to completely suberise (Lapwood & Adams 1973; Adams 1975; Adams & Lapwood 1978). However, this work suggests that tubers may be susceptible much closer to tuber initiation, whether through lenticel entry or by other means. Some research suggests that the pathogen may enter the tuber through direct penetration of the cell wall, rather than through non-suberised lenticels (Loria *et al.* 2003). There are two possible explanations for the increased disease suppression by treatments applied prior to tuber initiation. Firstly, these early treatments may either prevent or slow the possible direct pathogen entry through the suppression of thaxtomin toxicity. Secondly, as 2,4-D is slow to translocate from foliage to tubers, application prior to tuber initiation allows for a greater time period for the 2,4-D to accumulate within tubers before lenticels are susceptible to the pathogen.

Treatments applied soon after plant emergence and prior to tuber initiation resulted in both the translocation of 2,4-D to tubers as they were initiated, and effective disease control. This led to the hypothesis that treatments could be applied even prior to emergence, i.e. to the seed tuber before it was planted. Like very early foliar treatments, these seed tuber treatments were found to result in 2,4-D translocation to developing tubers, and were effective in controlling scab. However, the trials presented in chapter 4 were only exploratory, and further research is still required to optimise these treatments.

6.3 Hormonal effects of auxin

6.3.1 On tuber initiation

The increase in auxin within the plant from the applications of 2,4-D, either through foliar or seed tuber treatments, may affect tuber initiation. Dragicevic *et al*. (2008) found that treatments with IAA stimulated the formation of stolons, and, in shoots grown in the dark, the formation of tubers. Auxin levels in stolons have been demonstrated to increase dramatically prior to tuber initiation, and remain high during tuber growth (Roumeliotis *et al.* 2012). This suggests that auxin has a role in inducing tuber initiation, and an ongoing role in tuber growth. Treatments of 2,4-D may then, when applied prior to tuber initiation, result in earlier and possibly more even tuber initiation. Tuber initiation over a shorter period would reduce the period during which the crop was susceptible to disease, and subsequently reduce the period during which control methods such as targeted irrigation would need to be applied. Further studies are needed investigate the effect of 2,4-D application on the physical development of treated potato plants and tubers, particularly on the possible effect on emergence.

6.3.2 On plant/pathogen interactions

While auxins were not found to significantly affect the physiology of the potato tuber in relation to pathogen entry (Tegg *et al.* 2008), nor significantly affect the growth of the pathogen or its ability to produce thaxtomin A *in vitro* (McIntosh *et al.* 1981; Tegg *et al.* 2008), it is unknown what affect the applied auxin may have on the plant/pathogen interaction. However, auxin has been shown to induce pathogen defence systems associated with systemic acquired resistance (SAR) within potato plants (Zanetti *et al.* 2003), which is a possible explanation for the observed correlation between powdery scab and common scab disease severity. This may also have an effect on the auxin induced resistance, though likely to a lesser extent than the suppression of thaxtomin toxicity. Combined with the induction of SAR responses, auxin might also result in exudates from the plant into the soil that interact with the pathogen. Advances in analytical chemistry will make it easier to identify if auxin does induce the production of exudates. The effect of translocated 2,4-D on the population of the common scab pathogen either on the tuber surface or within lesions

has yet to be determined, and further research into this may provide a better understanding of the effect of auxin on the pathogen *in vivo*. Also, further studies into the possible inducement of SAR pathways by 2,4-D applications would provide insight into the possibility of 2,4-D providing control for diseases other than common scab.

6.4 Cultivar differences

The two varieties developed at different rates, particularly in the field trials. However, for ease of application both varieties were treated at the same time. Therefore some varietal differences in the efficacy of the treatments may be the result of the varieties varying in stages of development at treatment. It is likely that, for example, the two varieties began tuber development at different times, and this may explain the significant interaction between variety, spray rate and number of sprays observed in the first pot trial (Table 3.3), where later sprays appeared more successful in Desiree than Russet Burbank, the latter of which emerged slightly earlier.

For both trials in which the levels of 2,4-D in tubers at harvest was quantified, it was found that Desiree generally had lower amounts of 2,4-D than Russet Burbank. This suggests that the rate at which 2,4-D is translocated or metabolised within tubers differs between varieties. Burrell (1982) determined that the addition of IAA slowed translocation of 2,4-D to tubers, and varietal differences in endogenous auxin production, along with differences in growth and development may explain the differences in 2,4-D. While Russet Burbank had higher levels of 2,4-D in both trials, in one trial Russet Burbank had a significantly higher mean total tuber mass than Desiree, and in the other trial this was reversed. Therefore the amount of tuber tissue that the material is translocated to does not appear to have significantly influenced 2,4-D concentration within that tissue.

6.5 Disease pressure

While inoculum was bulked and incorporated into the potting mix following methods and using a strain of *S. scabies* that had been successful in previous years (Tegg *et al.* 2008) the apparent resultant disease pressure created by this inoculum in the pot trials was very low, with even control treatments having minimal lesions. It was suggested that the strain had a reduced pathogenicity or virility than it had in previous years, possibly due to excessive culturing, and as such the strain used in pot trials in chapter 4 was different to those used in chapters 2 and 3. The disease pressure did appear to increase, with control treatments in the daughter tuber pot trial having much higher disease severity than had been seen in the previous two seasons. However, the reduced disease pressure against tubers in the first two seasons of pot trials has demonstrated a trend towards foliar 2,4-D applications reducing disease severity under both low and higher levels of disease pressure.

6.6 Powdery scab disease

The repeated finding of a correlation between powdery scab common scab severity in the field trials was an interesting and unexpected result. While these two diseases are significant soil borne diseases of potato, there has been little research to date into the interaction between them. There are a number of possible explanations for this observed reduction in powdery scab severity as a result of the 2,4-D induced control of common scab. Firstly, common scab lesions may be used by the powdery scab pathogen as an entry point into the potato. The decrease in the number, size and depth of common scab lesions may therefore decrease powdery scab pathogen entry points. Secondly, treatments of potato plants with IAA have found to decrease the severity of late blight through inhibition of the pathogen (Martínez-Noël *et al.* 2001), suggesting that, while not inhibitory to *S. scabies* at the concentrations found within the tuber (McIntosh *et al.* 1981; Tegg *et al.* 2008) 2,4-D may be inhibitory to other potato pathogens. Thirdly, applications of IAA have been shown to increase the transcription of stress related proteins which may be involved in plant defence (Zanetti *et al.* 2003), and 2,4-D applications may therefore prime plant defence mechanism against the powdery scab pathogen.

6.7 Tuber slice assay

The tuber slice assay is a quick and reliable assay for determining tuber sensitivity to thaxtomins. Tegg *et al*. (2012) found that there was a correlation between the level of 2,4-D induced disease control and thaxtomin toxicity suppression measured using the tuber slice assay, and that this correlation held true for a number of other chemicals that appear to control common scab by suppressing thaxtomin toxicity. While the tuber slice assay is undertaken on a cut tuber surface rather than the tuber skin, where pathogen entry would take place, the study by Tegg *et al*. (2012) demonstrated that the assay was representative of the effectiveness of actual disease severity reduction.

In undertaking the study presented in this thesis it became clear that the time at which tubers were harvested and assayed was important. As chapter 2 showed, having the 2,4-D within the tuber from when it initiates is required for the most effective disease control. However, the assay was routinely performed on tubers that were harvested after senescence, and thus, when they were no longer susceptible to the pathogen. While this still gave an indication of the relative levels of toxin suppression achieved by treatments, and would likely provide a similar comparison between treatment rates, in future trials it would be more useful to undertake the assay on tubers harvested throughout the course of the trial. This data would have been particularly useful in determining the effectiveness of treatments applied at different dates. In trials such as these, tubers harvested after plant senescence would be less representative of the relative levels of disease control achieved by treatments. This is because the material within the tubers would have been metabolised for different lengths of time. However, in some experimental situations, the harvesting of tubers during the growing season is not possible. Additionally, determining the levels of 2,4-D within tubers after senescence is valid, as it allows for the comparison of tuber 2,4-D levels with the MRL.

6.8 Future development

The low rates required to induce the resistance to common scab of potato may mean that it will be difficult to move this research into development. 2,4-D is currently registered for application to potato crops in the USA, at a rate of approximately 647mg/L, and is being considered for registration in Canada, for the enhancement of the desirable red skin colour for fresh market potatoes (Waterer 2010). However, this rate is considerably higher than this study suggests is necessary for controlling common scab while minimising both phytotoxic effects and residue levels. As only a single application of a low amount of active ingredient is required, very little of the currently available commercial 2,4-D herbicides is required to spray entire crops with sufficient 2,4-D to provide disease protection. There is therefore less benefit to companies who produce and sell 2,4-D to go through the rigorous and expensive trials required to allow 2,4-D to be sprayed onto potato for common scab disease control, despite the interest shown in this control method from the potato processing industry in Australia. However, it may be possible to combine a 2,4-D seed tuber treatment with another, existing seed tuber treatment product, to create a unique treatment, the development and registration of which may be commercially viable.

While future development of the control of common scab with 2,4-D may not be financially viable for the agrochemical industry, the research presented in this chapter suggests that treatments of 2,4-D could provide a low cost disease control solution for growers, if regulation was approved. Additionally, it provides a model for future research, including into seed tuber treatments, further insights into the interactions both between the plant and the pathogen, and between common scab and powdery scab diseases, as well as further discussing possible mechanisms involving the suppression of thaxtomin toxicity with 2,4-D.

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